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BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLE, MASS.

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BIOLOGICAL BULLETIN

ECOLOGICAL SUCCESSION.

III. A Reconnaissance of its Causes in Ponds with Particular Reference to Fish.

VICTOR E. SHELFORD.

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I. Introduction.

In the preceding paper we presented certain facts concerning ponds, together with a statement of succession in the ponds at the head of Lake Michigan, without entering into its causes. Succession in ponds is due to many causes. It is only under the most favorable conditions that we can separate these causes one from another without long and careful investigation. The first attempts of ecologists in this line were considerations of the obvious general facts, such as the accumulation of organic detritus and the increased denseness of vegetation. We can give here a hint at the more specific changes in the ponds and the relations of these to fish. The subject is one for coöperative research. At present some of the workers and the necessary funds are not available, and the ponds are being destroyed rapidly. It is therefore improbable that the study can be carried further. This paper deals with the results of a preliminary investigation of the ponds for the purpose of learning something of the causes of distribution and succession of fish and other organisms in ponds.

II. The Present Character and Content of the Ponds.

The ponds with which we are concerned are shown on the map, p. 131, of the preceding paper of this series.[1] This map is essential to the understanding of the data of the present paper. The ponds here considered are an ecological age series, ecological age being determined by a study of amount of sand bottom, humus, etc., as shown in Table I. below. The physiographic history of the region is in full accord with the facts used in deciding age though in this case physiographic history is not essential to the decision. The pond designated as 1 is ecologically youngest, 14 the oldest, and the others intermediate. The measurements, analyses, and quantitative study were carried out on Pond 1, 5*c* (west section), 7*a*, and 14*b* of the map. Some qualitative records from the other parts of pond 5*c*, from 5*b*, and 14*a*, are included with those of the ponds in which the other work has been done.

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[1] See "Ecological Succession," II. Biol. Bull., Aug., 1911, pp. 127–151. These are errors in the pond numbers of this paper which should be corrected.

Page 132, line 13, for "56" read 58.

Page 133, Table I., last line, last column, for "15" read 52.

As we have already stated, the ponds which have been studied especially, are parts of the long sloughs which have been long enough isolated to show their efficiency in supporting the fishes which they now contain. The fishes found in the separated ponds are shown in Table XXI. (p. 17) of the present paper.

With the known habits of fresh-water fishes as a guide, the ponds have been roughly measured and area determined, depth and angle of the slope of sides measured, the character of bottom determined, sketched, and the areas of the different kinds estimated on the basis of the sketches, and the dissolved solids and gases of the waters have been determined by chemists. The plant and animal content of the ponds has been analyzed qualitatively and estimated quantitatively.

These results will be presented under the main heads of: (1) Physical Character, (2) Biological Content.

1. *Physical Character.*—(*a*) Topography. The chief topographic features are shown in Table I.

TABLE I.

SHOWING AREA, DEPTH AND SLOPE OF SIDES OF THE PONDS.

Pond.	Area in Meters	Greatest Depth in Meters	Average Depth	Area of Side Slope [illegible].	Area of Slight Slope
1	3,500	0.6	0.3	Much.	Little.
5c	3,500	0.9	0.5	Less.	Much
7a	25,000	0.9	0.5	Very little.	Much
14b	10,000	0.6	0.4	Very little.	Nearly all

The figures representing depth of water are the results of measurement, with estimation in the case of averages. Areas are results of rough measuring by pacing, counting rails in parallel railroads, etc. While portions studied differ in size, they present considerable uniformity of other features.

(*b*) Character of Bottom. The bottom is composed of pure sand, or sand more or less mixed with or deeply covered by humus. The sand or the humus has a considerable mixture of marl at some points in the younger ponds. Vegetation nearly always covers a pure humus bottom. *Chara* and bulrushes sometimes grow on sand and marl bottoms, but in such cases they are scattered and in the table such areas are included with bare sand, because such sparse vegetation does not interfere with the breeding of fish.

TABLE II.

SHOWING KINDS AND AREAS OF BOTTOMS.

Pond.	Area Sand in Square Meters.	Area Humus in Square Meters.	Depth Humus (Average) in Cm.
1	1,000	2,500	2.5 cm.
5*c*	50	3.450	20.0 cm.
7*a*	Very little.	Nearly all.	21.0 cm.
14*b*	None.	All.	24.0 cm.

It will be noticed that the area of sand is much less in the older ponds and the area of humus much greater, due to accumulation of the latter from the decay of vegetation. The depth of humus does not increase proportionately with age because it becomes more compact with time. With the exception of the first pond, the average depth of humus was obtained by dividing the average depth at the center by two. In the case of pond 1, there are large areas with only two centimeters of humus and two deep places which contain humus of considerable depth; we give only an estimate.

(*c*) The Dissolved Content of the Water. For a preliminary study of the dissolved solids of the water we have had a single analysis of the solids and four analyses of the gases made by chemists. (1) Solids. The small value of single analyses of solids is well known; sanitary analysts have pointed out the dangers arising from conclusions drawn from so little data. However, in this particular case, the value of the results is greater than in the case of single analyses of drinking water, because of the following conditions in and about the ponds.

(*a*) The ponds are without outlet and have no streams emptying into them.

(*b*) During rain they have little inwash because nearly all water must filter into them through sand; in case rain falls in such torrents as to actually run in from the sides, the area of drainage is small, being a strip not more than fifteen meters wide on either side of each pond. The ponds than are comparable to balanced aquaria and any variation of dissolved solids must be due in the main to the effect of organisms, of evaporation, and of renewal from rain.

It should be noted that these analyses were made at the end of the dry season and just at the close of the probable plankton maximum for the year.

TABLE III.

SALTS IN SOLUTION IN PARTS PER MILLION.

Analysis by Mariner and Hoskins (Chicago, Ill.). The collections were made on October 26, 1909. Wolt Lake contains all the species of fish of all the ponds and is added for comparison.

Pond.	1	5c	7a	14c	Wolf Lake.
Magnesium carbonate	84.6	111.9	38.2	77.1	96.8
Calcium carbonate	54.2	27.9	114.3	83.2	14.7
Calcium sulphate	149.6	146.6		18.5	174.2
Calcium chloride				11.4	9.2
Sodium sulphate	26.6	0.4	45.0		5.7
Sodium chloride	30.3	81.0	16.4	11.3	
Sodium carbonate			7.7		
Iron oxide	3.0	3.0	2.6	3.0	5.8
Silica	6.6	3.4	2.0	3.0	6.0
Total solids including those given in Table XVI	420.0	432.0	258.0	251.0	324.0

The table shows no unusal qualitative features. There is a notable decrease in total solids in the older ponds. This may be due to the fixing of the solids by organisms.

(2) Gases. The gas analyses were made with two facts in view: (*a*) Water may be abnormal in gas content, so as to make it impossible for fish to live (Marsh, '08; Juday and Wagner, '08; Birge and Juday, '11). (*b*) The eggs of all food fishes known to be in the ponds probably rest on the bottom or vegetation during incubation. Some fish remove the vegetation from the bottom; others deposit eggs on bare bottom; a few may attach eggs to vegetation.

To determine the general suitably of the ponds for the living places of fish, two determinations of the gas content of the open waters were made.

TABLE IV.

Oxygen in cubic centimeters per liter; collections 10–12 cm. below the surface of the open water.

Pond.	1	5	7a	14c
July 22, 1910	6.99	4.68	7.44	6.20
April 26, 1911	6.87	7.25	6.96	6.27
Average	6.93	5.97	7.20	6.23

The table shows an oxygen content in all the ponds, sufficient to support any of the fishes.

With reference to fish breeding places, the gas content of the water was determined on four occasions. To make collections in ponds *5c* over a sandy bottom required taking advantage of the sand areas made by artificial filling. Since there is little bare sand in ponds *7a* and *14b* the collections were made over the vegetation.

TABLE V.

Oxygen content in cubic centimeters per liter. Depth 35–40 centimeters. Sample collected at the bottom or among the upper two inches of branches of aquatic plants as indicated.

	Over Sandy Bottom.					Over Vegetation.			Bottom Materials Disturbed. Vegetation Removed in Summer				
Date.	6 27	7 22	4 26	5 10	Average.	6.27	7.22	Average.	6 27	7 22	4 26	5 10	Average.
1	6.57	6.37	5.91	6.32	6.28				3.34	5.91			4.62
5c	7.36	8.18	7.31	6.60	7.36				6.52	6.74			6.63
7a						4.42	2.52	3.47	0.00	0.00	6.26	3.36	2.40
14b						3.33	2.24	2.78	0.00	0.00	1.38	1.93	0.83

The table shows high oxygen content over sandy bottom, oxygen probably sufficient for most fish over vegetation, and no oxygen on the bottom where vegetation was removed in July and August.

TABLE VI.

SHOWING CO_2 CONTENT OF WATER.

Depth 35–40 cm. (1) Over sandy bottom. (2) Over vegetation. (3) Over bottom with vegetation removed.

	1		*5c*		*7a*		*14b*	
	1	3	1	3	1	3	2	3
June 27, 1910	0.0	4.1	0.0	0.0	4.4	21.0	3.3	6.6
July 22, 1910	0.0	0.0	0.0	0.0	2.5	9.6	2.4	4.0
Average	0.0	2.0	0.0	0.0	3.4	15.3	2.7	5.3

The water was alkaline at points showing no CO_2. The tables do not show the uniformity that might be expected. This may be explainable on the basis of the place of collection. *Chara*, for example, which was the plant removed from the bottom of ponds 1 and *5c*, grows on bottoms of mixed sand and humus or on a bottom covered with humus, and sufficient care was not taken in selecting places of collection, to make the differences here of importance.

There are apparently numerous factors which influence gas content (Birge and Juday, '11, p. 54). These are temperature, light as affecting photosynthesis, distance between point of collection and plants which are giving off oxygen and using CO_2, and direction and velocity of wind as affecting circulation of water. Birge and Juday (p. 55) state that European workers have noted marked diurnal changes in the amount of dissolved oxygen.

From three to four hours were required to make our collections. On June 27, the collecting began at pond 14*b* at 9:30 A.M., and ended at pond 5*c* at 12:30 P.M., temperature: pond 1, 26° C.; 5*c* and 7*a*, 27°; and 14*b*, 25°. Velocity of wind 6 miles per hour. This was a cloudy day. The sun broke through the clouds before the last collection was made. July 22 was a similar day. Collection began at pond 14*b* at 8:30 A.M., and ended at pond 7*a* at 12:00 M., temperature: pond 1, 25° C.; 5*c* and 7*a*, 26° C.; and 14*b*, 24°. During the forenoon, the sun came out several times, but exact record was not kept of the time or length of such periods of sunshine. All the other collections were made in full sunlight. Wind and temperature were as follows: April 26, temperature: 1, 13½°; 5*c*, 14½°; 7*a*, 15½°; and 14*b*, 14°; wind: 3 miles per hour. May 10, temperature, 23°; wind: 36 miles per hour. Just what effect distance from plants which were doing photosynthetic work has on gas content is not known. It is highly probable that collections made near to such plants would be different from those taken at a greater distance (Birge and Juday, '11, pp. 54 and 60).

The summer collections from pond 7*a* were taken from beneath the water lily leaves at the extreme east end where lilies have displaced the *Chara*.

Collections taken after scraping the vegetation from the bottom show various results depending upon the character of the bottom beneath the vegetation.

2. *Biological Content of the Pond.* (*a*) Qualitative Comparison. (1) Species of Plants and their Abundance. The qualitative differences in ponds as shown in Tables VII. and VIII.

(2) Growth Form of the Plants. Pond 1 is dominated by submerged plants. There are no broad-leaved shade producers.

TABLE VII.

SHOWING THE PLANTS OF THE CENTERS OF THE PONDS.

Data by Mr. G. D. Fuller. D = dominant; A = abundant; C = common; F = few.

Common Name.	Scientific Name.	Pond Numbers.			
		1	5*c*	7*a*	14*b*
Stonewort	*Nitella batrachosperma*	C			
Stonewort	*Chara* sp. 1	D	D	C	
Pondweed	*Potamogeton lucens* L	C	C	F	
Slender naias	*Naias flexilis* Rostk. & Schmidt.	C	D	F	F
Pondweed	*Potamogeton pectinatus* L.	A	?	C	F
Filamentous green algæ		F	F	F	A
Hornwort	*Ceratophyllum demersum* L.	A		F	?
Yellow water lily	*Nymphæa advena* Ait		F	C	F
Pondweed	*Potamogeton americanus* C. & S.		C	F	F
Water-milfoil	*Myriophyllum spicatum* L.			F	F
Bladderwort	*Utricularia vulgaris* L.			C	A
White water lily	*Castalia tuberosa* Greene			A	A
Water shield	*Brasenia Schreberi* Gmel			A	C
Stonewort	*Chara* sp. 2				C
Bulrush	*Scirpus validus* Vahl.				F
Duckweed	*Lemna minor* L				C

TABLE VIII.

SHOWING THE MARGINAL PLANTS.

Data by Mr. G. D. Fuller.

Roots Usually Submerged.

Common Name.	Scientific Name.	Pond Numbers.			
		1	5*c*	7*a*	14*b*
Bulrush	*Scirpus validus* Vahl	F	F	F	C
Cattail	*Typha latifolia* L			F	C
Mermaid weed	*Proserpinaca palustris* L				C

Roots Submerged at High Water.

Common Name.	Scientific Name.	1	5*c*	7*a*	14*b*
Sedges		C	F		
Pines	*Pinus Banksiana* Lamb		C	C	F
Shrubs (other than those below)			F	F	
Button bush	*Cephalanthus occidentalis* L			F	C
Willows	*Salix* spp			F	C
Swamp white oak	*Quercus bicolor*				F

Pond 5*c* shows the beginning of shade producers such as the water lily and of plants which reach above the surface of the water. Pond 7*a* has a large number of emerging plants. In one end of this pond there are many more of these than in the other. In pond 14*b* emergents are dominant.

(3) Animals. The different species of animals and their ar-

TABLE IX.

LEECHES.

Name.*	Pond Numbers.				
	1	5c	7a	14	30
Glossiphonia fusca Castle	*				
Erpobdella punctata Leidy	*	*	*		
Dina fervida Verrill	*	*	*	*	
Macrobdella decora Say		*	*	*	
Hæmopsis grandis Verrill		*	*	*	
Placobdella parasitica Say			*	*	
Placobdella rugosa Verrill			*	*	
Glossiphonia heteroclita					*
Hæmopsis marmoratis Moore					*

* For meaning of stars and letters see p. 11.

TABLE X.

SPHÆRIDÆ AND UNIONIDÆ.

Name	Pond Numbers.				
	1	5	7a	14	30
UNIONIDÆ:					
Lampsilis luteolus Lam.	*				
Anodonta grandis Say	*	*			
Anodonta marginata Say	*	*	*		
Anodonta grandis Footiana Lea.		*	*		
SPHÆRIDÆ:					
Musculium truncatum Lins.		*	*		*
Musculium securi Prime.			*	?	*
Musculium partumeium Say				*	?

TABLE XI.

SNAILS.

Name	Pond Numbers.				
	1	5	7a	14	30
Amnicola:					
Amnicola limosa Say	*	*	*	*	
Amnicola limosa cincinnatensis Lea.	*	*	*	*	
Amnicola limosa parva Lea			*	*	
Physa:					
Physa gyrina Say	F	F	C	C	C
Physa heterostropha? Say (?)				*	
Lymnæidæ:					
Planorbis bicarinatus Say	F	F			
Lymnæa humilis modicella Say	*	*	*		
Lymnæa obrussa Say	*	*	*		
Planorbis albus Mul	*	*	*	*	
Planorbis parvus Say	*		*	*	
Planorbis campanulatus Say	*	*	*	*	
Lymnæa reflexa Say	F	F	C	A	A
Planorbis deflectus Say			*		
Planorbis hirsutus Gld				*	*
Planorbis trivolvis Say				C	A
Segmentina armigera Say				*	?

TABLE XII.

CRUSTACEA.

Name.	Pond Numbers.				
	1	5c	7a	14b	30
Hyalella Knickerbockeri Bate	C	C	C	F	F
Eucrangonyx gracilis Smith		F	C	A	A
Mancasellus danielsi Rich					*
Asellus communis Say					*
Cambarus immunis Hagen	F	F	C	C	C
Cambarus blandingi acutos Girard				F	?

TABLE XIII.

AQUATIC INSECT LARVÆ AND NYMPHS.

Name.	Pond Numbers.				
	1	5c	7a	14b	30
May flies:					
Cænis sp.	*	*	*	*	
Siphlurus sp.	*	*	*	*	*
Callibætis sp.					*
Neuroptera:					
Chauliodes rasticornis Ram	*	*	*	*	*
Damsel flies:					
Lestes sp.	*				
Enallagma sp.	*	?	*	*	
Ischnura verticalis Say		*	?	*	*
Dragon flies:					
Tramea lacerata Hagen	*				
Celithemis eponina Drury	*				
Libellula pullcella Drury	*	*			
Gomphus spicatus Selys	*	*	*		
Leucorhinia intacta Hagen	*	*	*	*	
Anax junius Drury	*	*	*	*	*
Sympetrum rubicundulum Say		*			
Sympetrum sp.			*	?	*
Caddice worms:					
Geora sp.	*				
Leptocerinæ sp.	C	F			
Neuronia sp.			F	C	A
Diptera larvæ:					
Chironomid larvæ	*	*	*	*	*
Stratiomyid larvæ	*	*	*	*	*
Tanypus sp.		*	?	*	
Tipulid larvæ				*	?
Ceratopogon sp.					*
Hemiptera:					
Ranatra kirkaldyi Buen.	*	*			
Corixa sp.	*	*	*		
Ranatra fusca P.B.	*	*	*	?	?
Belostoma fluminea Say		*	*	*	*
Notonecta undulata Say		*	*	*	*
Buenoa platycnemis Fieb.			F	C	?
Plea striola Fieb.					*
Water striders:					
Gerris rufoscutellatus Lat.	*				
Gerris marginatus Say.	*	?	*		
Mesovelia bisignata Uhl.	*		*		

TABLE XIV.

HIGHER VERTEBRATES.

The fish are shown in Table XXI, page 17.

Name	Pond Numbers.				
	1	5c	7a	14b	30
Aromochelys odorata Lat.	*	*			
Rana pipiens Sch.	*	*	*	*	*
Chrysemys marginata Ag.	*	*	*	*	*
Malacoclemmys geographica Les.		*			
Diemictylus viridescens Raf.		*	*	*	*
Fiber zibethicus Linn.	?	?	?	?	?

The presence of the muskrat is indicated by the presence of holes, nests, tracks, etc., but none have been seen except in the oldest ponds.

rangement with respect to the ages of the ponds are shown in Tables IX. to XIV. Letters indicate relative abundance: F = few; C = common; A = abundant. The star is used to indicate presence where relative abundance has not been ascertained. For comparison, a fifth pond (No. 30) is added; this is older than the others in every respect and contains certain species of importance to fish which are not found in any of the others.

(4) Discussion of the Tables. The tables represent not only much careful collecting, but long experience with the common forms of the ponds. An inspection of the tables shows that there are differences in the species in the different ponds and that the differences are correlated with the ages of the ponds. For example, in the case of the leeches, Table IX., page 9, none of the species of the youngest pond is found in all of the ponds and none of the species of the oldest is found in the youngest. Accordingly as we pass from the youngest to the oldest we note that species disappear and are replaced by other species. The same will be seen to be true of the other groups. A similar relation is illustrated also where we have been able to estimate relative abundance. In some cases the number is greater in the older ponds; in others, less in the older ponds (*e. g.*, *Hyallela Knickerbockeri*, Table XII., page 10).

The case of the caddice worms and other aquatic insects which are placed in the water by the laying female, is of especial interest as the resulting distribution is probably either a matter of selection on the part of the female during the breeding season or striking elimination of all eggs laid in the ponds in which the larvæ are not found.

It is evident that ecological types (here represented by the various species) succeed each other as the ponds change with age. Succession is here as elsewhere, a succession of all, or at least a majority of the animals present.

(*b*) Quantitative Comparison. (1) Vegetation. Vegetation is evidently a good index of the content, or the relative numbers of the different species of plants and animals. In Table I., page 3, we note that more than two thirds of the bottom of pond 1 is covered with humus. Vegetation covers about 70 per cent. of the area. In pond 5*c* vegetation covers about 95 per cent. and in 7*a* about 99 per cent. of the area and in 14*b* 100 per cent. If the plants of each unit area were equal in volume, these percentages would represent relative volume also. More of the plants of the older ponds reach to the surface; plants are closer together in the older ponds. It is obvious from inspection that the volume per unit area is greater in the older ponds.

A single test was made with a large tow net. The net was drawn a distance of 40 feet in three of the ponds and the volume of vegetation torn off by the net was measured by displacement and reduced to terms of 100. This would give relative volume if all plants were torn with equal ease.

Finally Mr. G. D. Fuller and myself have made an estimate based on several inspections.

TABLE XV.

SHOWING MEASUREMENTS AND ESTIMATES OF RELATIVE VOLUME OF VEGETATION PER CUBIC UNIT.

Pond.	1	5*c*	7*a*	14*b*
On the basis of areas of vegetation	70	95	99	100
Tow net collections	14 c.c.	—	30 c.c.	100 c.c.
Estimate	20	40	60	100

(2) Plant and Animal Food. The plant and animal food in solution is expressed in a general way by the sanitary analysis. The results of a single analysis with the total carbonates added, are given in Table XVI.

Table XVI.

Showing Contamination of Pond 5*c* and Elementary Food Substances and Carbonates in All.

Single analysis, Oct. 26, 1909.

	1	5*c*	7*a*	14*b*	Wolf Lake.
Chlorine	18.4	49.7	9.9	14.2	16.3
Free ammonia	0.100	0.170	0.040	Trace	0.005
Albuminoid ammonia	0.125	0.150	0.175	0.250	0.200
Nitrites	Present	Trace	Trace	.....	Trace
Nitrates	0.160	0.030	0.030	0.040	0.060
Total carbonates	138.800	139.800	160.200	160.300	111.500

The chlorine content is regarded as a good index of the presence or absence of sewage contamination, excreta being high in chlorine compounds. 49.7 parts per million in pond 5*c* would indicate such contamination. Until very recently a house was located on the margin of pond 5*c*; the pond is still subject to contamination by domestic fowls.

Free ammonia is the final stage in the breaking down of proteids and appears also in animal excreta. It is used by plants and evidently plants consume it in proportion to their volume.

Albumenoid ammonia probably represents metaboloids in solution, because the water was filtered before determinations were made. Sewage is rich in such compounds and sanitary analysts have found that the number of bacteria is closely correlated with amount of albumenoid ammonia.

(3) Bacteria. Dr. P. G. Heinemann and Mrs. Class, of the Department of Bacteriology of the University of Chicago, very kindly made the counts of the bacteria. The results are given in Table XVII.

Table XVII.

Aerobic Bacteria per cc., Capable of Growing again at 20° C.

	1	5*c*	7*a*	14*b*	Wolf Lake.
October 26, 1909	158	48[1]	1,200	2,600	507
April 29, 1911	1,400	500	3,700	4,500	

[1] The number here does not correspond to the albumenoid ammonia, but may be partially accounted for by the fact that the bottle was accidentally opened near the surface. On April 26 a collection at the surface of the pond showed 350 (500 at bottom).

The table shows that the number of bacteria is greater in the older ponds, except in *5c* which is noncomparable because of contamination.

(4) The Plankton. The study of the plankton has been practically limited to the Entomostraca—the most important food of young fishes. The presence of a *larger* number of rotifers and protozoa, etc., is observable as we pass from the younger to the older ponds.

The number of Entomostraca in approximately 90 liters of surface water, to a depth of 10–12 decimeters, is given in the table below. It was thought best to simply dip the desired amount from the water while walking and strain the dippings through a bolting cloth strainer. After the first collection this was repeated in as uniform a manner as possible and Birge net collections were made at the same time for comparison. There was no great discrepancy in the results of the two methods of collecting, except in the case of Ostracoda in pond 14*b*. As compared with dippings, some Birge net collections showed less Ostracoda. Ostracoda were probably started from the bottom by the feet of the collector but were not by the drawing of the Birge net.

TABLE XVIII.

THE NUMBER OF ENTOMOSTRACA IN 90 LITERS OF WATER.

	1	5*c*	7*a*	14*b*
September 3, 1909	556	...	539	2,773
November 13, 1909	200	106	397	350
March 26, 1910	42	40	12	00
May 31, 1910	3,497	1,014	4,368	3,600
July 22, 1910	160	200	520	6,480
April 26, 1911	1,250	150	140	525
May 10 1911	100	800	125	5,125
Total of 6	5,249	2,310	5,562	16,080
Average of 6	874	385	927	2,680

The table shows that with the exception of pond 5*c*, which is probably noncomparable because of contamination, the older ponds contain most Entomostraca except in early spring when conditions are somewhat reversed.

A large quantity of plankton in old ponds has been noted for several years in connection with class work. For comparison with the ponds under consideration we have studied Wolf Lake, and two small ponds near it. The younger of the small ponds will be designated as I. and the older one, II. They differ (with the exception of the margin vegetation) in much the same manner as do ponds 1 and *7a* of the series of special study. While Wolf Lake is not strictly comparable to the others, it is ecologically the youngest, because of its greater area of bare bottom. The collections (made Sept. 3, 1909) were four in number in Wolf Lake, four in pond I., two in pond II., one half from the open water, and one half from among vegetation. Several collections were made Apr. 30. The numbers given are the averages of all collections made on those dates. They were net collections made in as uniform a manner as possible.

TABLE XIX.

COLLECTIONS SHOWING DIFFERENCES IN NUMBERS OF ENTOMOSTRACA CORRELATED WITH DIFFERENCES IN ECOLOGICAL AGE.

Date	Order	Body of Water		
		Wolf Lake.	I.	II.
September 3.	Copepoda	149	128	918
	Cladocera	64	96	3,936
	Ostracoda	0	8	81
	Total	213	232	4,115
April 30	Copepoda	3,750	1,500	26,600
	Cladocera.	200	12,500	500
	Ostracoda.	400	0	1,500
	Total.	4,350	14,000	28,600

This table shows the same features as the preceding.

(5) The Larger Animals. Little has been done in estimating the relative number or volume of the larger animals in the different ponds. A general idea is given below in Table XX. This

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[1] THE RELATIVE NUMBER OF THE ORDERS OF ENTOMOSTRACA IN THE COLLECTIONS OF 1910.

Order.	Pond Numbers.			
	1	5	*7a*	14
Copepoda.	4,523	1,167	4,774	4,520
Cladocera.	371	273	421	1,057
Ostracoda.	25	40	202	4,073
Total	4,919	1,480	5,407	10,850

The deficiency in 5c is due mainly to small numbers of copepods.

is based on the general impression which has been acquired in taking classes to these and other ponds of similar character several times per year during six years. Secondly, by taking the time required to make a representative collection from the different ponds. On the basis of this experience, the figures given in the table are thought to be very conservative. That there is a far greater number of animals and a greater volume of animal substance in the old ponds is very easily demonstrated to any one by inspection.

Table XX.

Showing an Estimate of the Relative Numbers of the Chief Items of Fish Food in the Different Ponds.

	1	5*c*	7*a*	14*b*
Entomostraca	32	15	35	100
Chironomid larvæ	80?	80?	80?	100?
Sphaeridæ	0	30	50	100
Gilled snails	20	30	50	100
Pulmonate snails	10	30	50	100
Amphipods	50	70	90	100
Decapods	10	30	50	100
Insects	40	60	90	100
Fish	80	100	70	30

Previous to being drained pond 14*a* should be rated at 70 for fishes.

While the results here presented are not such as to justify conclusions concerning details, we may state that the amount of life per unit volume unquestionably increases as the ponds grow older, at least up to stages like 14*b*. Qualitative differences are shown in the Tables VII. and XIV., and the total number of species recorded in each pond is about the same, the actual quantity is far greater in the older.

III. The Causes of Succession of Fish.

A discussion of succession must be made with reference to all the organisms of the habitat, or at least a large number of them considered in mass. Succession of one group of organisms taking place without the succession of others in the same environment seems improbable. A discussion with reference to fish must take other organisms into consideration.

1. *Statement of the Problem.*—A clear understanding of the problem at hand will perhaps be facilitated by a careful state-

ment of the question before us, after which we shall discuss the available data with reference to the relations of fish to the different ponds, from the standpoint of their area, their depth, minerals and gases in solution and finally the available food for young and adults. Competition, living place and breeding place of the fish will be discussed as fully as data will permit.

TABLE XXI.

DISTRIBUTION OF THE FISH AND THEIR RELATION TO BOTTOM.

The letters and numbers at the heads of the columns refer to the various isolated parts of ponds. The star indicates the presence of the species; B, that very young specimens were found in numbers and the species bred in 1909, 10 or 11. The nomenclature and bottom preference data are after Forbes and Richardson, '08.

Common Name.	Scientific Name.	Ponds				Bottom	Bottom.
		1	5	7	14	Preferred (F. & R., '08).	Present or Absent with Fish.
Large-mouthed black bass	*Micropterus salmoides*	B	. .	. . . .	. . .	Rock and sand.	Present.
Blue gill. .	*Lepomis pallidus*	B	.	. . . .	. . .	Rock and sand.	"
Blue-spotted sun fish.	*Lepomis cyanellus* . .	B	. . .	. . . .	. . .	"	"
Pumpkin seed	*Eupomotis gibbosus*	B	. . .	. . .		"	"
Warmouth Bass.	*Chaenobryttus gulosus*. . .	B	.	.		Mud.	Muck present
Yellow perch.	*Perca flavescens*	B	B	.	. .	. . . .	. . . .
Chub suckers.	*Erimyzon sucetta*	B	B	.		Rock and sand.	In part.
Spotted bullhead. . .	*Ameiurus nebulosus*	*	B	B		. . . .	. . . .
Tadpole cat.	*Schilbeodes gyrinus.*	*	B	B	. .	Mud and sand.	In part, muck present
Pickerel.	*Esox vermiculatus*	B	B	B	. . .	. . . .	. .
Mud minnow. . .	*Umbra limi*	*	B	B	. .	Mud (Abbott).	Muck present
Golden shiner.	*Abramis crysoleucas*		B	B	. . .	Mud.	"
Yellow bullhead.	*Ameiurus natalis*		.	*	.	"	"
Black bullhead	*Ameiurus melas*			B	B	"	"

The problem of the causes of succession may be stated in two ways:

(*a*) Involving interpretation: Why are the pioneer fishes of a pond succeeded as the pond grows older, by fishes of different habits?

(*b*) Independent of interpretation: Why are the fishes of pond I. not in the older ponds and the fishes of the older ponds not in pond I., when the channels between them have been open until the past few years?

2. *The Cause of Succession—Environment.*—(*a*) Area of the Ponds. A comparison of Table I., page 3, with Table XXI., page 17, and a comparison of Table I. of the preceding paper with the map (p. 131 of the preceding paper) show that most of the fishes are in ponds of all the available areas of the region, with the exception of several species which are confined to pond I., and which, on account of their numbers, could find no advantage in such close quarters. Evidently no part of the answer lies in the matter of size.

(*b*) Depth of the Ponds. A comparison of the records of depths given in Table II., page 4, with Table XXI., page 17, shows a situation parallel to the one with reference to area. Species are in ponds of various depths and are absent from ponds of depths the same as and greater than the ones in which they are found. These ponds are shallower than the waters which many of the species commonly occupy. The matter of depth does not seem to be of importance in the answer to the question.

(*c*) Minerals in Solution. The minerals in solution in the different ponds on October 26, 1909, are given in Table III.

(1) Qualitative Differences. The minerals represented in the analysis are those normal to waters inhabited by fish and probably important to fish. No zinc, lead, aluminum, silver, or copper, metals highly poisonous to fish (Marsh, '10), were found and there is no reason to expect their presence at another time of the year.[1] From the qualitative standpoint there is no reason to assign importance to minerals in solution.

(2) Quantitative Differences. The total solids given in Table III., p. 5, lie between the two extremes given by Marsh, '10, as probably not affecting fish and as "normal" for waters which are known to support fish in numbers. He gives 484 parts per million for the Potomac River and 242 for other fish waters. Nor is a very great seasonal variation to be expected, because most of the animals live through the winter and the vegetation disintegrates very slowly, especially through the cold weather,

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[1] Because of the small amount of inwash, this set of ponds affords an unusual opportunity for the study of the effect of a varying amount of vegetation on the chemical composition of the water. For a statement of the salts tied up by plants see Hefter-Ewert, '00, page 410.

and in the spring its place is taken by new vegetation as rapidly as the decomposition of the old takes place.

From our knowledge of the composition of river water inhabited by all the fish, before and after the floods, no great importance could be assigned to minerals, even though the complexion of the analyses changed with the season. However, no positive conclusion could be drawn without careful study of the *behavior reactions* of fish to minute quantities of salt.

(*d*) Gases. The results of gas determination are given in Tables IV., V., and VI., pp. 5 and 6. Tables IV. and V. show the gas content of the open water, above the vegetation and sandy bottom, to be sufficient for fish in all the ponds. Juday and Birge, '11, p. 130, state: "König found that he could keep fish (kind not specified) in water which contained 2.95 c.c. and 1.38 c.c. of dissolved oxygen per liter without any apparent ill effects. Thörner found that a fish epidemic was caused by the absence of free oxygen. Hoppe-Seyler and Duncan state that trout which were kept from one and a half to two and a quarter hours in water having only from 0.98 to 1.71 c.c. of oxygen per liter showed marked signs of dyspnœa. Paton, in experiments on young rainbow trout, found that a fall in the amount of dissolved oxygen below one third of the normal amount, *i. e.*, below 2 c.c. per liter of water, is prejudicial and generally fatal. Some individuals however, were able to sustain life for long periods in water which contained only minimal traces of dissolved oxygen.

"Knauthe found that carp kept for an hour and twenty minutes in water which contained 1.33 c.c. of oxygen per liter, did not show any signs of dyspnœa, while others became dyspnœic in water containing from 2 c.c. to 3.1 c.c. of this gas."

Birge and Juday state also that Mackinaw trout have been taken from waters with 1 c.c. per liter. Fish diseases are said to be more prevalent in low oxygen content (Knauthe, '07). In this case there is no reason for assigning importance to the oxygen content of the open waters frequented by fish, and this factor is nearly uniform in the different ponds. The oxygen content of the bottom is of great importance and will be discussed later in connection with breeding.

(*e*) Temperature. A single set of readings taken in the late afternoon of a warm sunny day showed less than 1 degree of difference between the different ponds and the readings were not repeated.

(*f*) Excretory Materials in Solution. Dacknowski ('06) (see Cowles, '11) found that certain unknown water soluble substances present in bog water are poisonous to plants. Colton ('08), and authors cited by him, found that the excretory products of animals are toxic to the producer, and sometimes to other organisms. This is a physiological basis for succession. Knauthe states that the effect of fish on their environments is important, but little of definite character is known concerning it.

(*g*) Food. The food of the fishes from these ponds has not been studied, but knowledge of the food habits of the same species was acquired from the study of literature, especially the work of Forbes and Hankinson. The species found in the ponds being known, each pond was inspected with reference to the things eaten by each fish species. Forbes gives the percentage which each item constituted in the individuals which he studied.

(1) Qualitative. The method of obtaining the results consisted in adding Forbes' percentages ['80, p. 38] for the different items of food for each species found in each pond. For example, take the food of lake specimens of the perch. These were found to have eaten fish food existing in pond 1 as follows: decapods rated at 14 per cent.; unidentified fish, 50 per cent.; Acanthopteri, 8 per cent., giving a total of 72 per cent. Pond 1 contains 72 per cent. of the food of lake perch; Cyprinidae rated at 28 per cent. do not occur (see Table XXII). For the youngest individuals (under one inch) of all the species, all the ponds are qualitatively equal. Hankinson's data on Walnut Lake species show that all our ponds are about qualitatively equal for the fish which he considers.

An inspection of Table XXII, p. 21, shows that in no case are the fish *confined* to the place where their food is *qualitatively* best, in fact, as a rule, the fish are in the pond where the food is qualitatively *poorest*. The available data on the food of fishes shows that the fish eat food *available* where they live, rather than that their *distribution is due to the presence or absence of certain food species.* Excluding students of the food of animals, the idea that food determines distribution is commonly, though erroneously, held.

TABLE XXII.

QUALITATIVE EXPRESSION — VALUE IN FISH FOOD.

* indicates presence of the species being considered. The averages are not averages of the figures given here, but of all Forbes' items taken separately; their number is given in the last column.

Species.	Size.	Pond Numbers.				No. of Items Averaged.
		1	5^c	7^a	14^b	
		Per Cent	Per Cent.	Per Cent.	Per Cent.	
Micropterus salmoides.	1–2 in.	98	100	100	100	
	2–4 in.	100	100	100	100	
	Adults.	36	42	34	21	
	Average.	*86	88	86	85	5
Lepomis pallidus.	1–3 in.	100	100	100	100	
	Adults.	81	81	81	81	
	Adults	60	60	80	80	
	Adults.	91	91	91	91	
	Average.	*88	88	92	92	6
Lepomis cyanellus . . .	1 in	100	100	100	100	
	1–4 in.	96	96	96	100	
	Adults.	58	71	71	58	
	Average.	*88	91	91	89	5
Eupomotis gibbosus	1–4 in.	100	100	100	100	
	Adults.	81	81	87	87	
	Average.	*95	95	96	96	4
Chænobryttus gulosus.	All.	*100	100	100	100	
Perca flavescens. . . .	1–3 in.	100	100	100	100	
	3–4 in.	76	76	76	76	
	Adults.	72	100	92	90	
	Adults.	56	61	64	60	
	Average.	*83	*89	88	88	6
Erimyzon sucetta	All.	*100	*100	100	100	
Ameiurus nebulosus and *mela*	Various					
	young.	100	100	100	100	
	Adults.	80	93	73	69	
	Average.	*90	*96	*86	*84	3
Schilbeodes gyrinus.	Various					
	young.	100	100	100	100	
	Adults.	66	66	78	78	
	Average.	*83	*83	*88	89	2
Esox vermiculatus . .	1¼ in.	100	100	100	100	
	Adults.	40	40	42	42	
	Average.	*70	*70	*71	71	2
Umbra limi.	Adult.	*33	*33	*33	68	
Abramis crysoleucas	Adult.	86	*86	*86	86	
Ameiurus natalis.	Adult.	60	64	*64	64	

(2) Quantity of Food. The quantity of food, like the quality, is one of the reasons assigned for the distribution, migration, and extinction of animals. Although my data on quantity of food in the ponds is not as good as that on quality, a comparison is presented in Table XXIV.

In the case of the young fishes, the table follows from a comparison of the tables of Forbes with our own on Entomostraca. The quantity of food for the youngest individuals of all species is practically that of the Entomostraca: Pond 1, *32;* pond 5*c*, *15;* pond 7*a*, *35;* pond 14*b*, *100*. For the adults and young from one inch to four inches in length, an estimate of the quantity of food in each pond for each species has been made by averaging the ratings of the principal articles of food given for each species by Forbes.

TABLE XXIII.

METHOD RATING PONDS. *Ameiurus natalis.*

Diet According to Forbes.	Rating in Table XX.			
	1	5*c*	7*a*	14*b*
Insects, 30 per cent	40	60	90	100
Fish, 34 per cent	80	100	70	30
Decapods, 17 per cent	10	30	50	100
Average	43	63	70	76

The ratings being only estimates, a more accurate method is unnecessary.

An inspection of Table XXIV shows that the distribution of fish is not correlated with *quantity* of the foods known to be eaten by that species of fish in other localities. The fish are frequently *found only* in the ponds where the food is *least abundant* and no fish is found where its food is most abundant. Are the fish the cause of the deficiency of their own food? To answer this question Wolf Lake and the small ponds were studied. Wolf Lake contains many more fish than any of the other bodies of water thus far mentioned, but as it is a large body we cannot compare it with the ponds. Pond I. (see p. 15), which has been artifically separated from Wolf Lake, contains few fish—*Abramis crysoleucas*, *Umbra limi*, and *Ameiurus nebulosus* are the only species and these appear not to be numerous. Pond II. contains

Table XXIV.

Quantity of Food; the Rating of the Ponds for the Different Species.

* shows distribution of fish.

		1	5	7*a*	14
Micropterus salmoides	Young.	*37	45	72	100
	Adult.	*45	65	60	65
Lepomis pallidus	Young.	*37	38	62	100
	Adult.	*33	50	77	100
Lepomis cyanellus	Young.	*30	41	60	100
	Adult.	*42	62	78	82
Eupomotis gibbosus	Young.	*37	45	71	100
	Adult.	*37	50	73	100
Chænobryttus gulosus	Young.	*37	43	71	100
	Adult.	*53	73	83	76
Perca flavescens.	Young.	*40	*48	71	100
	Adult.	*40	*58	70	86
Erimyzon sucetta	Young.	*50	*70	90	100
	Adult.	*50	*70	90	100
Ameiurus nebulosus and *melas*	Adult.	*32	*55	*65	*82
Schilbeodes gyrinus	Adult	*45	*65	*90	100
Esox vermiculatus	Young	*54	*61	*61	76
	Adult	*31	*42	*60	57
Umbra limi	Adult	*35	*48	*68	97
Abramis crysoleucas	Adult	20	*31	*48	96
Ameiurus natalis	Adult	43	63	*70	76

Abramis, *Umbra* and *Esox vermiculatus* all fairly abundant. It is evident that pond I. contains fewer fish per unit volume, still it has less Entomostraca. Evidently consumption by fish does not greatly affect Entomostraca.[1] The condition with respect to Entomostraca is paralleled by other elements of fish food.

(*h*) Competition of Species. On this point we have been able to secure almost no data. The golden shiner is absent from pond I. So far as the conditions are concerned, it should be present in numbers. It is an important article of diet for many of the fishes found there, which suggests that it has been eliminated by the other fishes.

3. *Relative Importance of the Breeding Activities and General Activities.*—The activities will be separated into general and breeding.

(*a*) General Activities. This will be taken up with reference to the depth of water, kind of bottom and surrounding vegetation with which the fish are commonly associated, according to the various writers cited.

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[1] My statement (Shelford, '10) to the effect that the amount of fish food consumed is about the same in all the ponds of our series (I., 5*c*, 7*a*, and 14*b*) was based on 14*a*, which has been drained. Table XX., page 16, shows that was incorrect.

Micropterus salmoides.

Vegetation of the pond weed zone (Hankinson, '07, p. 213); 3 to 25 feet—plants: *Potamogeton*, *Naias*, *Myriophyllum*, *Elodea* (Davis in Hankinson's Report).

Generally prefers still and sluggish waters (Forbes and Richardson, '08).

Lepomis pallidus.

5 to 15 feet of water, patches of *Potamogeton* and other aquatic plants (Jordan and Everman, '02).

Pond weed zone, 3 to 25 feet of water (Hankinson, '07).

Lepomis cyanellus.

Shoals where plants were abundant; bulrushes and aquatic types (Hankinson, '07).

Small streams (Forbes and Richardson, '08).

Eupomotis gibbosus.

Plant covered shoals—0 to 3 feet (Hankinson, '07).

Chænobryttus gulosus.

Shallow mud bottomed ponds or lakes (Jordan and Everman). Still water, muddy bottom, plenty of vegetation (Meek, '08).

Deep pools and quiet water (Henshall, '03).

Perca flavescens.

Chiefly an inhabitant of the pond weed zone; seldom found in less than two feet of water (Hankinson, '07).

Gregarious; moderate depths of streams and ponds (Henshall, '03).

Erimyzon sucetta.

Limited to places where vegetation was abundant (Hankinson, '07).

Ameiurus nebulosus.

Loves mud; lives in weedy ponds and rivers without current (Jordanand Everman, '02).

Fond of mud; weedy ponds and rivers without current (Forbes and Richardson, '08, p. 206).

Pond weed zone, shallow water at night (Hankinson, '07).

Schilbeodes gyrinus.

Common in dense vegetation of the shallow, almost stagnant water of bays.

Hides under stones and logs (Hay, '94).

Esox vermiculatus.

Situations with most aquatic vegetation (Jordan and Everman, '02).

Preference for quiet muddy water; weedy streams (Forbes and Richardson, '08).

Grassy streams and muddy bayous (Henshall, '03).

Umbra limi.

Never seen swimming in the open water; only where aquatic plants formed a dense growth in shallow water (Hankinson, '07).

Bury themselves in a hole in the mud scooped out with the tail; rest there at an angle of 45° with the tail down and the head barely protruding (Abbott, '70).

Mr. Dwight L. Gardner has shown by experimental studies in our laboratory that they avoid strong light.

Abramis crysoleucas.

Common in all places where there are many water plants (Hankinson, '07).

Muddiest and apparently most uninviting holes (Hay, '94).

Ameiurus natalis.

Generally frequenting the pond weed zone from which it went into shallow water at night. Young in shallow water with dense vegetation (Hankinson, '07).

Streams with muddy bottom (Forbes and Richardson, '08).

Ameiurus melas.

Small ponds with muck bottom (Jordan and Everman, '02).

A comparison of the data above with that in Table I., p. 3, and Table XXI., p. 17, shows that the large mouthed black bass, the blue gill, the warmouth, the perch and the yellow and spotted bullheads are not in water of the depth which they prefer in other localities. The other fishes are better located as to the depth of the water.

The large mouthed black bass, the blue gill, the perch, and the spotted and yellow bullheads are found chiefly in the pond weed zone of Walnut Lake. This is characterized by plants that do not reach the surface. They are *Chara*, hornwort, bladderwort, water millfoil, water weed, slender *Naias*, pond weeds, etc. (Davis in Hankinson, '08). These same plants grow also in the bays and coves in company with the water lily and other emerging plants.

Ponds 1 and 5*c* are dominated by submerged plants. Here the perch, bass and sunfish mentioned above are associated, with the same species and the same *growth form types* as in Walnut Lake. The bullheads are found common in the ponds in which the submerged and emerging vegetation are mixed, and which contain the greatest number of *species* of the pond weed zone of Walnut Lake. It seems impossible to draw any conclusion here as to the relation of these species to either species or growth form in plants. The whole subject is one for investigation. A comparison of Tables II., p. 4, and XXI., p. 17, shows that black bass, the sunfishes and pumpkinseed are found only where a considerable area of their preferred bottom is present.

Mud and muck are evidently not distinguished in the tables of Forbes and Richardson ('08) and it is not possible to make much use of their data here for this reason. We have noted in the preceding paper that the chubsucker prefers coarse bottom materials. If muck is included with mud (Forbes and Richardson, '08) with the exception of the warmouth and chubsucker, all are well placed. The chubsucker, the mudminnow, and the golden shiner, tadpole cats and the bullheads avoid strong light, and their association with dense vegetation which results, brings them into relations with *bottoms of fine material, e. g., muck*, because they support dense vegetation (Pond, '05).

(*b*) Breeding Activities. We give below all that has been found regarding the location of nest and eggs.

Micropterus salmoides: Sterile bottom of clay, sand or gravel, fibrous roots of the parrot feather preferred to others (Titcomb, '07, p. 10 of separate, fide Stranahan); (*b*) blackened roots of waterfoil 1 to 2½ feet of water, bulrush shoals in 12 to 15 inches of water, among conspicuous growth of bulrushes, eggs on roots (Hankinson, '07, p. 214); (*c*) leaves of trees, gravel; used when artificial fibrous nest was present (Reighard, '05, p. 48); (*d*) sand, gravel preferred, mud, clay, or surface of plants in absence of these (Henshall, '03); (*e*) gravel, clay or mud from which all foreign materials have been removed (Smith, '07, p. 247).

Lepomis pallidus: Barren shoals; bottom pure marl or marl and sand, bottom of marl or gravel; water 5 inches to 2 feet; marl bottom with bulrushes (Hankinson, '07, p. 212).

Lepomis cyanellus: Swamp loosestrife, black bottom, 1 foot of water; marl, marl and sand, also roots (Hankinson, '07, p. 210).

Eupomotis gibbosus: (*a*) Sand bottom; 1 to 2 feet of water; sand bottom; marl and sand bottom, scant bulrush growth; marl bottom, bulrush covered (Hankinson, '07); (*b*) sand and gravel bottom not infrequently on roots (Reighard in Gill, '05, p. 513); (*c*) clear water; sand and gravel bottom (Henshall, '03).

Perca flavescens: (*a*) No nest; bare sand and gravel (river), among aquatic plants (Abbott, '75); (*b*) stones, vegetation, other submerged objects or loose in water—no nest (Smith, '07, p. 252).

Ameiurus nebulosus: (*a*) Stove pipe, etc., 4–5 feet, sand, under cover, in 3–24 in. of water (rarely more than 24 in.) (Eycleshymer, '07); (*b*) gravel and aquarium bottom (Kendall, '02; Smith and Harron, '02).

Schilbeodes gyrinus: In tin can, marl bottom, 3 feet of water (Hankinson, '07).

Umbra limi: Stuck to aquatic plants (Ryder, '86).

TABLE XXV.

SHOWING THE RELATION OF KNOWN BREEDING HABITS OF FISH TO CONDITIONS IN THE SERIES OF PONDS.

Name of Fish	Breeding Bottom Present with Fish	Depth of Water Commonly Selected in Inches.	Depth over Breeding Grounds Present with Fish in Inches.
Micropterus salmoides	Sand.	12–36	0–18
Lepomis pallidus	Sand.	5–60	0–18
Lepomis cyanellus	Sand.	12	0–18
Eupomotis gibbosus	Sand.	12–24	0–18
Perca flavescens	Sand and vegetation.	Quite near the shore.	
Ameiurus nebulosus	Sand under cover.	3–24[1]	0–18
Umbra limi	Vegetation.		

The data on breeding habits as summarized in Table XXV. show clearly that the *distribution* of the species whose breeding habits are known is *correlated with the distribution of the conditions* necessary *for breeding.*

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[1] Greater depth evidently rare. Apparently *usually in very* shallow water.

While our tables show that there is considerable bare bottom in the pond 5*c*, there is good evidence that this is largely due to building of the road and of the Lake Shore and Mich. Southern R. R. which separated this pond from the others and from the lake and probably excluded fish since 1851. The exposures of bare sandy bottom which are due to natural causes are usually not covered with more than six inches of water.

Turning to the perch which is abundant here we note that the eggs are extruded in the open water or vegetation as well as over terrigenous bottom. Terrigenous bottom is less necessary than to the other food fishes.

Turning to the spotted bullhead we note that the nests are probably usually made in water shallower than any of the other fishes. Only one specimen has been taken from pond I.; they are numerous in pond 5*c* and 7*a*. There are some old logs and stumps and a very narrow zone of bare sand in 6 in. and less of water in these ponds. This is commonly shaded by vegetation.

In connection with oxygen content we note that it is greatest in 5*c* where the first four species of Table XXV. do not breed. However, this pond must be regarded as in a measure noncomparable because of contamination and small amount of plankton.

The low oxygen content on the muck bottoms of the older ponds, at depths used by the fishes present in pond I., and absent from these older ones, certainly is a sufficient reason for their absence, though it is not to be expected that this is the sole cause. It is apparent also that *A. nebulosus*, which is present in the older ponds, not only breeds in shallower water but also has superior means of aerating the eggs (Smith and Harron, '02).

Succession of fish then becomes succession of *breeding conditions* and *breeding mores.* While the major factors as indicated here are related to depth and bottom, there are doubtless others.

IV. General Discussion.

There is great danger of error in dealing with such complex problems when compilation is necessary and especially when the point of view of the compiler differs from that of the original investigator. To illustrate principles and methods we have relied upon compilation far more than could otherwise be justified. Still certain facts and relations appear to be clearly indicated by this reconnaissance. These will be roughly grouped under the heads quantitative, economic and general.

1. *Quantitative.* As has been pointed out in the body of the paper, the quantity of living material in the form of plankton, invertebrates, and vegetation increases as a pond grows ecologically older. In our data there are two exceptions to this which must be noted: First the greater number of Entomostraca in the younger ponds in early spring and the lesser number in pond 5*c* on all occasions. The greater number in the early spring is not easily explained but may be due to the better conditions on the bottom where the eggs, etc., of the plankton Entomostraca are found. Possibly the larger areas of clean bottom prevent their being buried and shut away from the effect of the sun's heat, oxygen, etc.

Pond 5*c* is, as we have indicated, probably not comparable on account of the contamination; also plankton production is measured in Crustacea and Marsh ('03) has pointed out possible errors in this method. A study of all the plankton constituents might show a different relation of 5*c*. Here, however, low plankton content is associated with little CO_2 (Birge and Juday, '11).

The rooted gross vegetation secures necessary salts from the soil and Pond ('05) pointed out that it increases plankton because the foods absorbed from the soil are added to the water when the plants decay. Our results are then in full accord with those of Pond. (See also Birge & Juday, '11, Knauthe, '07, p. 578.)

The greater number of large invertebrates appears to be generally closely related to the amount of gross vegetation. Nearly all such animals cling in vegetation and many of the species found in the older ponds use the vegetation as a means of reaching the surface for air, of avoiding strong sunlight, and as breeding places. The majority of such animals place their eggs into or upon the plants. Gross vegetation is also thickly covered with minute organisms which afford food for many animals.

It is probable that the amount of *rooted* vegetation in isolated ponds may be taken as an index of plankton production. It appears that this must be true on the basis of the conclusions of Pond ('05) no matter what factor is of greatest importance in controlling the quantity of plankton. Johnstone ('08) pointed out that the plankton production follows Liebig's law of minimum—*i. e.*, quantity is determined by the food substance present in minimal quantity. If rooted vegetation is the controlling factor a deficiency in one food substance in the soil would show itself in the rooted vegetation and through this affect the plankton production of the pond.

The question of the general application of the principle of quantitative increase with age is important. It seems probable that in all bodies of water with small outflow organisms increase with age because, in addition to the effect of rooted vegetation, inwash continuously brings food substances which are tied up if not carried away by extensive outflow.

Experimental study of the *quantitative* problem is possible on the basis of such a set of ponds as those at the head of Lake Michigan. From such a set all the organisms can be transplanted and most of the conditions duplicated where closer control would be possible than in the natural ponds. There appears to be no difficulty in such experimental study except that it requires extensive facilities and institution or government support. Such ponds as ours and such ponds as may be constructed with them as a basis give promise of throwing more light on the factors controlling the quantity of life than do the large and complex bodies of water.

2. *Economic.*—The writer has no practical knowledge of fish culture and only the knowledge *which has been acquired by reading* some of the characteristic literature. Apparently the economic problems in fishes are concerned with questions of the preservation of fishes in natural waters, and their increase and maintenance against the removal for food, which makes them of economic importance. With these ends in view efforts have long been made mainly to increase fish by increasing food supply, to care for fish during the critical reproductive season by artificial hatching and pond culture, and to decrease enemies by destruction of objectionable fish and fish parasites. The preservation

of the fish environments has received little or no attention. Laws have been enacted to prevent the pollution of waters, but these have been enforced but rarely.

In practice the importance of the breeding season has been recognized by the culture workers but appears to have received little attention from the point of view of the preservation or cultivation of fish breeding places in the natural waters. Clark ('10) is one of the few who have emphasized breeding grounds. The main emphasis has been laid on nutrition (Knauthe, '07, Chap. IV.).

Our data indicate that the breeding interests and the feeding interests of still water food and game fishes are *distinctly antagonistic*. Birge ('10) pointed out that where the quantity of plankton is great and the fish food accordingly great, the oxygen content is low at the bottom and the water accordingly unsuited to the production of certain of the best food fishes. Knauthe (p. 579) states that a large fish productivity in a pond is commonly indicated by large amount of gross vegetation, but says also that the general statement that such ponds are always good producers of fish cannot be made. This indicates that there are other factors. He makes no mention of breeding and does not state the practice of pond owners as relating to the breeding. In standing and sluggish water, the problem of the balance between the food supply and the fish present seems relatively unimportant. Since feeding conditions of desirable food fishes grow better with time at the expense of the breeding conditions, the major problem is that of the *balance between feeding and breeding conditions*. It appears that such balance might be maintained easily if we had an adequate knowledge of the environmental relations of the fish. Definite knowledge as to spacing of nests in nature should give data as to breeding area required per capita by fish. With such knowledge at hand, together with the existing knowledge of food habits, it should not be difficult to maintain adequate breeding areas adjacent to good feeding areas within our waters both public and private.

3. *General.*—We have noted the aspects of the quantitative and economic problems which our data enable us to discuss. The remaining indications of the reconnaissance are those related to factors governing distribution and methods of study.

The study of factors governing distribution of fish and other animals has never been reduced to an adequate working basis. The problems are indeed complex, but the difficulty has arisen in part from two causes, namely, (*A*) the lack of knowledge of the activity which takes place within the narrowest limits (Shelford, '11[3]), and (*B*) lack of recognition of the important factors and features of the environment.

The conclusions of workers on distribution often seem to have been to the effect that the food relations of fishes should stand as first in importance, as factors of distribution. Hankinson ('10) states that the pond weed zone, the living and feeding place of the fish of Walnut Lake, is probably the most important habitat. Our evidence on the same species points clearly to the breeding grounds. Indeed much careful work must be done before broad generalization should follow, but it is evident that here as in birds (Merriam, '90; Adams, '08) and in the tiger beetles (Shelford, '07, '11[3]) the breeding place and the breeding activities are the most important. (Reighard, '10, and citations.) Is variation in nest building real or only apparent because we do not know the most important factors and seize upon details wholly unessential to fish? What are the laws governing the *mores of species?* Experimental work correlated with field observations can answer these questions, and it is at this point that contributions of lasting value can be made. The first step in the necessary work of raising natural history from its present state of vagary is to determine what activity takes place within narrowest limits and which is least modifiable in as many groups of animals as possible.

The second difficulty—lack of recognition of the important and unimportant in environments—is one which we have emphasized before.

The ecologist often uses vegetation as an index of conditions. There is objection to this. Investigators have seen that the same species of animals are not always associated with a given species of plant. Indeed, *species* of plants cannot often and perhaps usually be taken as an index of the environmental conditions of animals, especially in water, because *species* of plants are not necessarily an index of conditions. The physiological condition of plants is the important thing and is commonly indicated by growth form (superficially but not finally) which is the index of internal physiological state induced by the surrounding conditions. Plant formations are the expression of the conditions of existence for the plants of a definite area. The formation is the fundamental unit of the ecology of communities and carries with it *no consideration of species whatever.* Identical or similar formations often do not have a single species in common. As we have pointed out before, species are of importance only in so far as their ecological constitutions are specific characters. It is not *species* of fish that we are to expect to be associated with species of plants, but *mores* of fish with *growth form* in plants or with plant formations. Furthermore, relations to vegetation which are of importance are to be expected primarily in connection with *breeding.*

Objection to the use of vegetation as an index of conditions, due to misapprehension, is to be expected. However, when the theoretical probabilities are understood, we have not the data in the case of fish, with which to determine whether or not *growth-form* and *mores* are associated. The subject is one for special experimental and observational investigation.

In connection with the problems of animal behavior, this point of view opens up a field wherein the rôle of the different environmental conditions in the control of behavior may be studied in nature as well as in experiment. As a background for the study of all aspects of behavior the point of view here presented seems to offer decided advantages.

Comparative study of behavior from this point of view has been impracticable because of a lack of knowledge of environments. Until we can acquire a knowledge and a nomenclature that shall be generally understood the worker must write extensive descriptions of the environment, and is likely to emphasize details which are of little importance.

The activities of an animal (behavior) are of great economic importance, they determine distribution. The relations of the *behavior problems and the distribution, the quantitative and the economic problems seem especially intimate, so that the investigation of any one from this point of view must contribute to all* as well as to bring about a better unification and organization of biological science as a whole.

V. Summary of Tentative Conclusions.

1. The quantity of bacteria, plankton, vegetation and large animals increases as a pond grows older.

2. Terrigenous bottom and oxygen content decrease as a pond grows older.

3. The distribution and succession of fish are not determined by kind of food; kind of food eaten is determined by the availability in localities suitable in other respects.

4. Fish are not necessarily present where food is quantitatively greatest.

5. The food and game fishes here considered are closely associated with their breeding conditions to the neglect of depth of water, food, etc.

6. Low oxygen content on breeding grounds is a sufficient cause for their absence from the older ponds.

7. Conditions outside the breeding season are probably of secondary importance in the success of fish in a given locality.

8. The food interests and breeding interests of the food and game fish here considered are decidedly antagonistic. The former continually encroaches upon the latter.

9. Successful fish culture in ponds and small lakes depends upon the maintenance of balance between the breeding and feeding conditions.

10. Animal succession in ponds is due to an unused increment of excretory and decomposition materials which causes an increase in vegetation, a decrease in O_2, on the bottom and a general change in surrounding conditions, all primarily affecting *breeding*.

11. Succession of species is the result of stability of the *mores* of species concerned; when mores are flexible species do not succeed one another but continue with changes in behavior and physiological characters.

Hull Zoölogical Laboratories,
University of Chicago,
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tically all nucleus. The corpuscles on the other hand have lost their nuclei wholly. Between these extremes we have various stages of chromatin reduction in the development of the specialized Metazoan tissues. The maturation divisions in ova and sperm, the bodily extrusion of chromatin observed on the part of blood-cells, etc., he regards as illustrations of the process.

THE RESERVE OF FOOD IN TREES

Preston and Phillips (Forest Quart., 1911) agree with the common view that starch is the principal form in which reserve food is stored in trees. They doubt that cellulose is able to act at all as a reserve material. The maximum contained reserve for deciduous trees occurs about the time the leaves fall, and during the next few weeks there is a decided reduction in its amount. The sugar content in trees remains pretty constant except for an increase in spring during the unfolding of the buds.

ALTERNATION OF GENERATION IN FLORIDEÆ

Lewis (Bot. Gaz., Mch., 1912), by artificial plantings of tetraspores and carpospores of *Polysiphonia* and some other genera of red algae gets experimental results supporting the general conclusion that tetraspores produce only the sexual plants and carpospores only the tetrasporic plants. In no instance was an exception found to the rule, although a considerable number of plantings developed to maturity. Tetraspores from a given individual produced male and female plants in approximately equal numbers. It is also concluded that no greater growth vigor comes to the carpospores over the tetraspores because of the double number of chromosomes contained by them.

RELATION OF THE PROTOPLASM OF ADJACENT PROTOPLASTS

Thoday (Ann. Bot., 1911) undertakes to throw light on the relation that exists between protoplasms of contiguous cells, by an examination of the relation between the parasite, *Cuscuta*, and its host. She finds that there is no direct protoplasmic connection between the cells of *Cuscuta* and the host, but that the phloem cells of the parasite haustoria apply themselves to the sieve plates of the phloem of

Ecology

ECOLOGICAL SUCCESSION OF PLANTS AND ANIMALS

Sh 11.31.

Shelford (Biol. Bull., Dec., 1911) concludes a series of papers dealing with the biological succession in ponds at the head of Lake Michigan. The following are some of the conclusions reached by the author as the result of this series of interesting studies:

1. The quantity of bacteria, plankton, vegetation, and large animals increases with the age of the pond.

2. Terrigenous bottom and oxygen content decrease with the age of the pond.

3. Fish tend to adapt themselves to the type of food rather than to become distributed or furnish successions in accordance with the type of food. They are not necessarily most abundant where food is greatest.

4. Small oxygen content of older ponds will account for absence of fish from them.

5. Conditions outside the breeding season are probably less important than those of this season in determining the success of fish.

6. The conditions most favorable to the normal feeding of fish are not only different from those most favorable to breeding, but are even antagonistic; and the former tend to encroach on the latter. ment, and the preservation of balance between the breeding conditions and the adult life-conditions.

7. Animal succession in ponds is due to an unused increment of excretory and decomposition products which causes increase in vegetation; a decrease in oxygen at the bottom; and a general change in the conditions affecting breeding.

8. Succession of particular species, rather than the continued dominance of some when they once become dominant, results from the inflexibility of their standards of demands in accordance with the changing conditions.

CHROMATIC REDUCTION IN CELL DEVELOPMENT

~~Rohde (Zeit. Wiss. Zool., 1911) undertakes to show that a marked characteristic of the differentiation and maturing of cells is the reduction of chromatin of the nucleus. He suggests, as illustrative of this, a series with bacteria at one end and the red blood-cells of mammals at the other. The bacteria he considers as prac-~~

THE CENTRAL NERVOUS SYSTEM IN TERATOPHTHALMIC AND TERATOMORPHIC FORMS OF PLANARIA DOROTOCEPHALA.

C. M. CHILD AND E. V. M. McKIE.

The study of the nervous system in the teratophthalmic and teratomorphic forms of *Planaria dorotocephala* was undertaken by the junior author of this paper at the senior author's suggestion. The results of this study were accepted as a thesis for the Master's degree by the Department of Zoölogy of the University of Chicago. Since the results of the work are of considerable interest and since Miss McKie was prevented by various circumstances from preparing the paper for publication, the senior author has undertaken, at her express request, to revise her manuscript for publication and to add some figures from her slides; he has also added a section on the various methods by which the teratophthalmic and teratomorphic forms have been produced and has extended somewhat the scope of the discussion of the results.

The primary object of the work was to determine the general form and the degree of development of the cephalic part of the central nervous system in these abnormal forms as compared with normal animals. The observations concern chiefly the teratomorphic forms since these represent a more extreme departure from the normal type and afford more definite and striking results.

The animals for sectioning were anesthetized with weak alcohol before fixation in hot Gilson's fluid or sublimate. Sections were cut ten micra in thickness. Frontal and sagittal as well as transverse sections were made, but all the figures are drawn from transverse sections since these show the essential features most clearly.

All figures of sections were drawn to the same scale with the camera. They are designed to show, first the general form of the nervous system and second, the general relations between fiber tracts and cells. The cells are represented merely by small circles or ovals and the fiber tracts are filled in with dots, except where a distinct commissure or nerve is concerned; there the direction of the fibers is indicated. Non-nervous structures are not shown except in the case of the alimentary tract, which is diagrammatically indicated where it is present in the sections figured.

I. THE EXPERIMENTAL PRODUCTION OF TERATOPHTHALMIC AND TERATOMORPHIC FORMS.

The senior author has given the names "teratophthalmic" and "teratomorphic" to certain types of head which appear under certain conditions in the regulation of pieces of *Planaria*. The teratophthalmic head (Child, '11*a*, pp. 278-9; '11*c*) is one in which the eyes show some departure from the usual structure or arrangement, but the head is otherwise normal in form. The teratophthalmic forms may be divided into several groups according to the character of the eyes, for these may be "abnormal" in position, size or number or the pigment cups may show the most various degrees of fusion (*e. g.*, Fig. 6 below).

The teratomorphic heads (Child, '11*c*) represent a more extreme departure from the norm. In these the abnormalities involve not only the eyes but the shape of the head and the position of the auricles. The teratomorphic head usually possesses a single median eye and the auricular sense organs appear on the front of the head, either separate (Figs. 10 and 16) or more or less completely fused (Figs. 19 and 23). In the senior author's earlier work on *Planaria* the teratomorphic heads were not separated from the teratophthalmic (Child, '11*a*), but as the degree of experimental control in the production of these forms increased it became desirable to set these peculiar forms apart as a distinct group and to give them a name.

It is possible, as the senior author has shown in various papers (Child, '11*a*, '11*c*, '11*d*), to control experimentally by a number of different methods the production of these forms. In general they are the result of conditions which decrease the rate of the dynamic processes below a certain level determined by existing conditions which is necessary for the production of normal animals. With the proper experimental conditions they can be produced from any region of the planarian body and from pieces of any size above a certain minimum, which varies with region of the body, physiological condition, age, nutrition and external conditions. A regional factor does, however, exist (Child, '11*a*): in pieces of a given length the more posterior the level within a single zoöid, the greater the frequency of the abnormal forms.

Thus far it has been possible to control experimentally the production of teratophthalmic and teratomorphic as well as anophthalmic and headless forms (Child, '11*c*) in the following ways: first, under standard conditions of temperature, nutrition, etc., pieces above a certain length with anterior ends at a certain level of the body will produce normal wholes, shorter pieces will produce teratophthalmic forms and still shorter pieces teratomorphic, anophthalmic and headless forms as the length decreases. Second, in pieces of a given length from a given region, under uniform conditions of temperature, nutrition, etc., stimulation to motor activity increases the frequency of normal animals, while lack of stimulation increases the frequency of teratophthalmic, teratomorphic, anophthalmic and headless forms. Third, in pieces of a given length from a given region of animals of the same size the frequency of abnormal and normal forms varies with differences in physiological age (Child, '11*b*) and with differences in nutrition. Fourth, in pieces of a given length from a given region of animals of the same size and as nearly as possible in the same physiological condition a variety of external factors such as low temperature, metabolic products in the water, dilute alcohol, ether, chloretone, potassium cyanide, etc., will increase the frequency of abnormal forms and it is possible to control to a certain extent the type of abnormal form, both through the length of the piece and the intensity of the experimental factor. On the other hand, the frequency of normal forms in a given set of pieces can be increased by good nutrition, by high temperature and probably also by certain stimulating drugs, though as regards these last the results are complicated by the fact that in many cases the stimulating effect of drugs is of relatively short duration and is followed by a depression.

But whatever may be the results of more extended experiment, the facts already established demonstrate that the normal and the abnormal forms described represent differences in the dynamic processes which are primarily purely quantitative. The temperature experiments illustrate this point very clearly. In a given set of pieces higher temperatures increase the frequency of normal, lower temperatures that of abnormal forms. The effect of the anesthetics and the other external factors mentioned above is probably also primarily quantitative.

In these cases then different morphological characteristics appear as the result of primarily quantitative changes in the dynamic processes in the organism. This fact is of considerable theoretical importance, since it can mean nothing else than that form, structure, localization, number and even presence or absence of parts may be determined by purely quantitative changes in external factors, *i. e.*, by changes which alter primarily the rate and not the character of the dynamic processes.

Certain external characteristics of the head region of the abnormal forms, viz., the position and number of the eyes and auricles, indicate that the cephalic ganglia of these forms must show considerable departures from the norm. The question as to how the form and structure of the central nervous system may be altered by these quantitative changes in the dynamic processes is one of interest from various points of view. The data presented below give a partial answer to this question and so form a contribution to our knowledge of the dynamics of morphogenesis.

The method used for obtaining the teratophthalmic and teratomorphic forms described in this paper was that of cutting pieces of a certain length, determined by previous experiments of the senior author, from the middle region of the body of large, well fed worms and allowing them to undergo regulation at about 20° C. This method was used merely because it is the simplest. Teratophthalmic and teratomorphic heads develop on pieces of greater length from the middle region of the body, *i. e.*, the posterior region of the first zoöid (Child, '11*a*, '11*e*) than from any other region. This makes it possible to use relatively long pieces and the preparation and handling of the material is therefore less difficult. Of course the abnormal heads can be obtained from still longer pieces if regulation occurs at low temperatures, but the length of time necessary for regulation in such cases is a disadvantage. Abnormal heads produced by the action of anesthetics and by various other conditions were not included within the scope of the present investigation. It is not improbable that comparison of the heads produced by different conditions will show more or less characteristic differences in the nervous system.

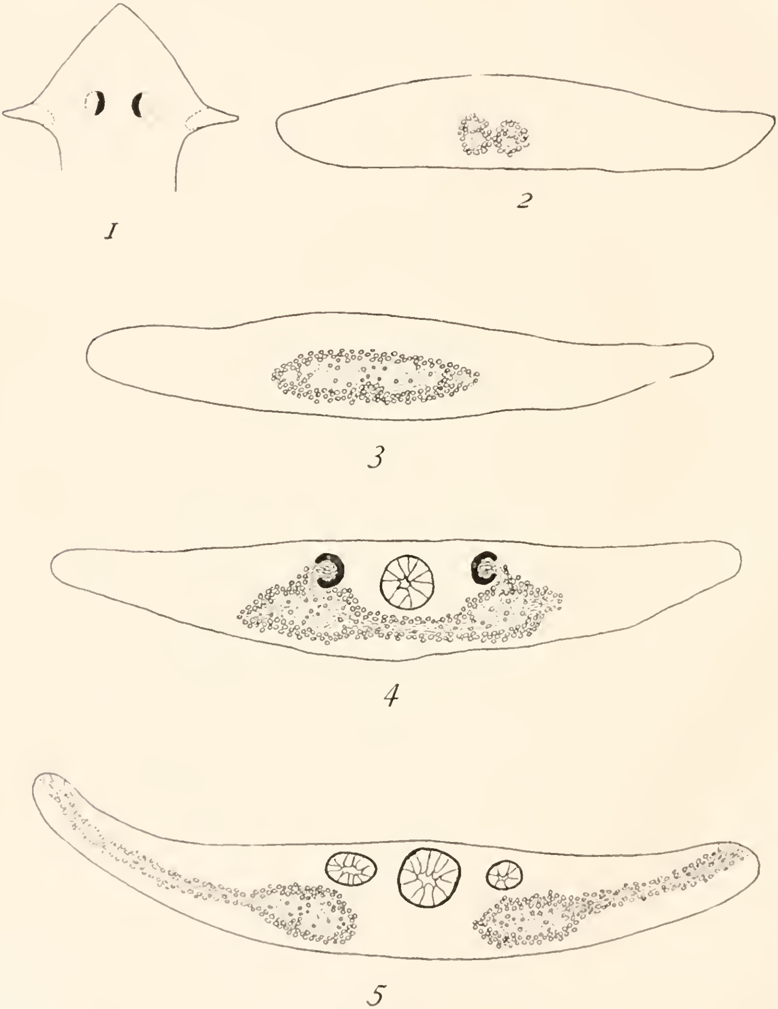
In all cases described the pieces were kept for at least two weeks after section. After this length of time the new head is well developed and those cases in which the teratomorphic head does not remain teratomorphic but redifferentiates into a head of normal shape have already undergone this further regulation or show unmistakable indications of it. The teratomorphic heads which persist as such for two weeks at 20° C. almost never show any further changes.

II. The Head of the Normal Animal.

The form and the chief external features of the normal head of *Planaria dorotocephala* are shown in Fig. 1. The unpigmented areas of the eyes and the very slightly pigmented sensory regions of the auricles are indicated by dotted lines. Except for these the dorsal surface of the head is usually rather deeply and uniformly pigmented. From the ventral surface the outlines of the cephalic ganglia and nerve cords are indistinctly visible in the living animal.

Figures 2–5 show transverse sections of the nervous system at different levels of the head region. Fig. 2 is from a level about half way between the eyes and the tip of the head and shows four nerves extending to the anterior head region. Further anteriorly these nerves break up and become less distinct as they are distributed. Fig. 3 shows the ganglia at about one fourth of the distance from the eyes to the tip. They consist of fiber tracts including a few cells and surrounded by many others. At this level the chief fiber tract shows indications of a beginning separation into right and left halves. On each side of the chief tract is a small tract separated from it by cells: these two small tracts are cross sections of nerves which pass to the anterior regions of the head.

Figure 4 shows the ganglia at the level of the eyes. The two masses lie some distance apart and are connected by commissures. The pigment cups of the eyes open laterally and the optic nerves pass in a dorso-ventral direction. Between the eyes the median anterior branch of the alimentary tract appears.

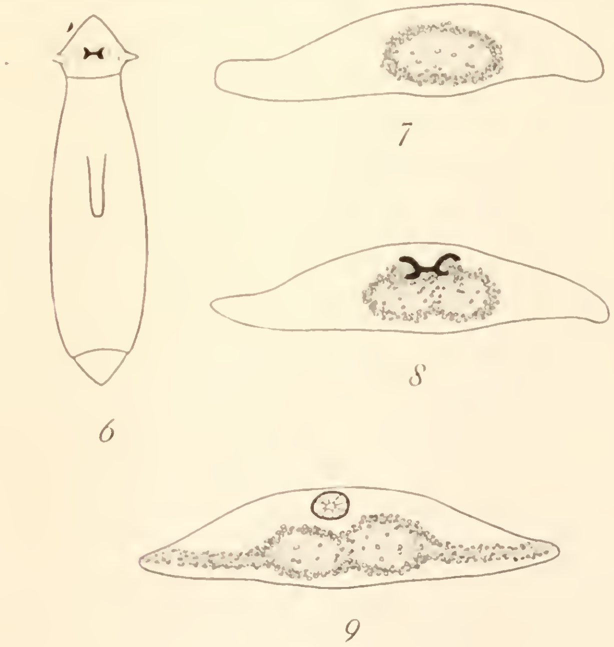


Figs. 1–5.

Figure 5 is from a section at the level of the auricles. Here there is no distinct commissure between the ganglionic masses. It is quite possible, however, that they are not entirely disconnected as they appear in the figure; some nerve fibers may extend across the space between them. The nerves passing to the auricles appear in the section and the alimentary tract lies on the dorsal side. Posterior to this level the nervous system consists of the two main nerve cords, each composed of a fiber tract including some cells and surrounded by others and giving rise to nerves and commissures at various levels.

III. Teratophthalmic Heads.

Of the various types of teratophthalmic heads only those which show partial fusion of the optic pigment cups were examined. The forms with unequal or unsymmetrical eyes constitute a somewhat different type of teratophthalmia and require a more extended investigation: moreover, the partial fusions of the eyes lead through all possible stages to the single median eye of the teratomorphic head.



Figs. 6-9.

Figure 6 shows the outline of the body and the condition of the eyes in one of the teratophthalmic heads sectioned. The two optic pigment cups are symmetrically situated but lie closer together than in normal animals and are united by a continuous band of pigment.

In Fig. 7 a transverse section of the nervous system from about the posterior fourth of the preocular region is shown. It consists of a single fiber tract surrounded by cells and without any trace of division into right and left halves. Comparison with Fig. 3 which is from about the same level in the normal animal shows a marked difference in form. Fig. 8 shows the level of the eyes. The difference between this and Fig. 4 from the normal animal is striking. In Fig. 8 the fiber tract is partially divided into right and left halves, but the two parts are close together instead of being widely separated and connected by a long commissure as in Fig. 4. In Fig. 9 a section at the level of the auricles is drawn: much the same differences from the normal (Fig. 5) appear here. The two ganglionic masses are closely connected, while in the normal animal they are widely separated.

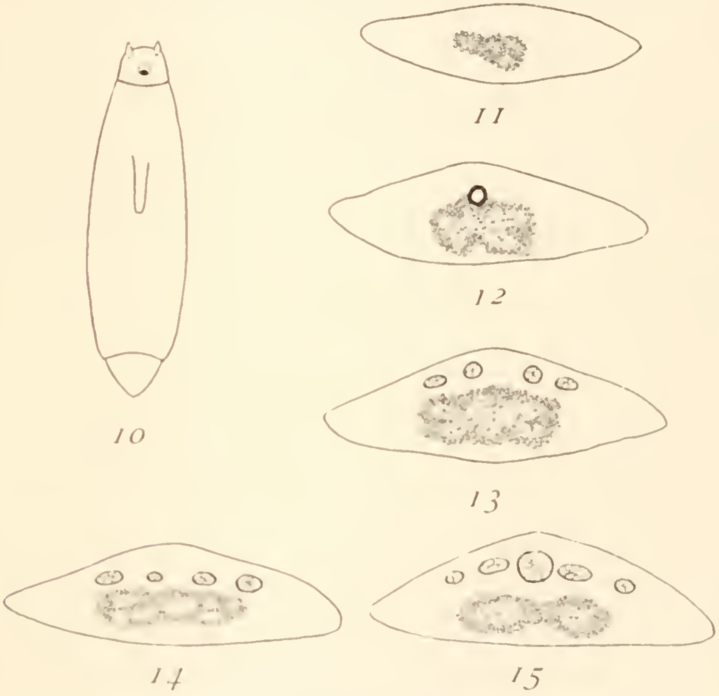
The figures from this teratophthalmic head show one other point of interest. The individual from which the sections were made was much smaller than the full grown animal of Figs. 2–5. Figs. 2–5 and 7–9 are drawn to the same scale and comparison shows at once that the ganglia are almost as large in the teratophthalmic as in the normal animal. This is a general characteristic of physiologically younger as compared with older and of smaller as compared with larger animals. In the small young animal the nervous system is always of relatively large size and in small animals which result from the regulation of pieces the same is true, except in the more extreme abnormal types, where the nervous system is often small. Thus as regards the development of the nervous system as well as its rate of metabolism during development (Child, '11*b*) the animal formed by regulation resembles a young animal.

In other teratophthalmic individuals with partially fused eyes the general form of the ganglia was found to be much the same as in the case described and the degree of fusion or separation of the ganglia corresponds rather closely with the degree of fusion or separation of the eyes. In these forms then the eyes serve to some extent as an index of the condition of the nervous system.

IV. Teratomorphic Heads.

1. The first case to be described is shown in Fig. 10. Here the auricles appear on the front of the head and extend anteriorly. The anterior margin of the head between them is slightly rounded instead of pointed as in the normal animal. In the median line is a single eye.

Sections of the head region of this animal are shown in Figs. 11–15. The eye is situated almost at the extreme anterior end of the ganglionic mass and a few sections anterior to it the nervous system appears as in Fig. 11. Here four fiber tracts surrounded by cells are visible and are evidently nerves to the anterior regions of the head. The conditions at the level of the eye are shown in Fig. 12. The single optic pigment cup opens anteriorly instead of laterally, as the adjoining sections on the slide show, and it is farther from the dorsal surface of the head and more nearly imbedded in the ganglionic mass than in



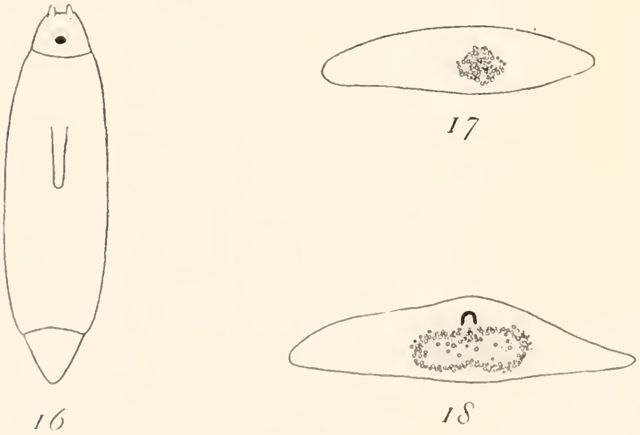
Figs. 10–15.

the normal animal (Fig. 4). The ganglionic mass itself is somewhat irregular in form and shows no trace of a division into symmetrical right and left portions.

Figure 13 shows the condition of the ganglia six sections (sixty micra) posterior to the eye. Here the fiber tracts show indications of a symmetrical arrangement, but this arrangement is widely different from the normal. Three sections farther posteriorly the fiber tracts are still more broken up, as shown in Fig. 14. The level of this section is approximately the posterior end of the anterior new tissue of the regenerated region. Fig. 15 shows a section sixty micra posterior to the level of Fig. 14, *i. e.*, in the old tissue: here the nervous system appears in the usual form of two ganglionic ventral cords, which, however, are much less widely separated than in the normal animal at this level.

2. The animal sectioned is shown in Fig. 16. The head is much like that in Fig. 10, but the auricles are somewhat closer together. A single median eye with a rather large pigment spot is present.

Figures 17 and 18 show sections of the head. Fig. 17 is a section a short distance anterior to the eyes, about the posterior fourth of the preocular region. Fig. 18 shows the level of the eyes. The single pigment cup appears in the figure to open ventrally, but the opening is actually antero-ventral in direction. The ganglionic mass is distinctly double, *i. e.*, more like the



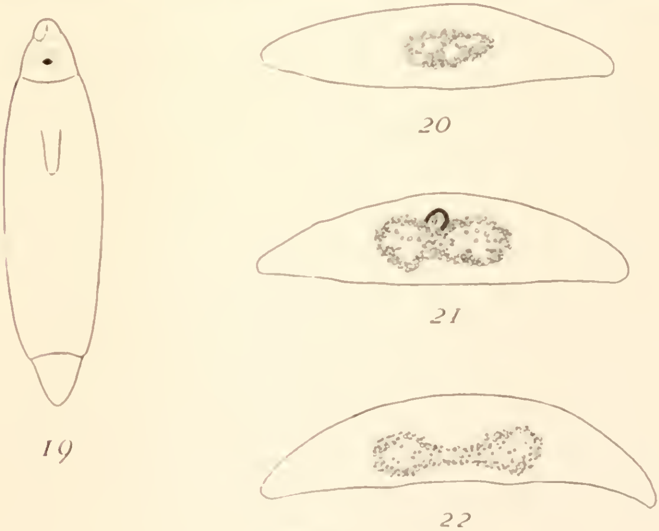
FIGS. 16–18.

normal than that of Fig. 12. Two nerves, one from each portion of the ganglionic mass, pass to the optic cup.

Here, as in the preceding case, the eye is situated near the extreme anterior end of the ganglionic region instead of a considerable distance posterior to it as in normal forms. Posterior to the eye the form of the ganglionic mass continues much the same as in Fig. 18 to about the posterior end of the new tissue, where the right and left portions become more distinctly separated with a commissure between them and then pass into the two nerve cords.

In general form the nervous system is much less abnormal in this than in the preceding case. The chief differences from the norm are the anterior position of the eye on the ganglionic mass and the partial fusion of the two ganglia for a considerable distance posterior to the eye.

3. As indicated in Fig. 19, this case shows a somewhat extreme form of teratomorphism. The two auricles are fused at the tip of the head, though the sensory areas are in large part separate. A single median eye is present as in the preceding cases.



Figs. 19-22.

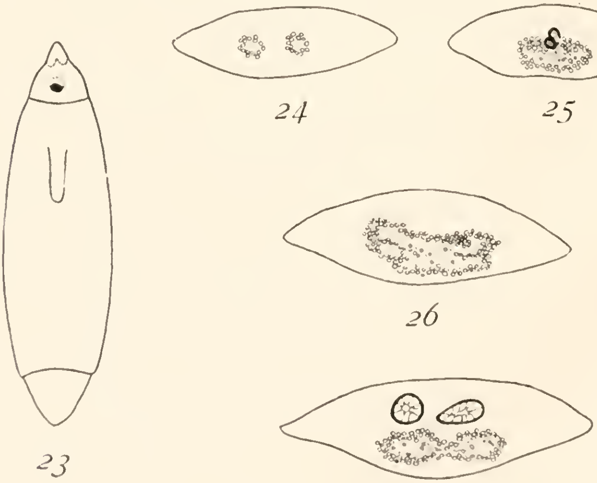
Fig. 20 shows a transverse section of the nervous system at the level where the nerves to the front of the head arise; this is about one fifth of the distance from the eyes to the tip of the head. Five fiber tracts unsymmetrically situated are indicated in the section.

In Fig. 21 the level of the eye is shown. The optic pigment cup opens antero-ventrally and toward the left side and is connected by a nerve with the left side only of the ganglionic mass. The latter shows a distinct division into right and left halves.

Near the posterior end of the regenerated region the nervous system possesses the form shown in Fig. 22 and a short distance posterior to this level and in the old tissue the two nerve cords become separate except for an occasional commissure.

In this case the eye, though median in position, evidently belongs to the left half of the ganglionic mass and the nervous system is much less abnormal than in Case 1. As in the other cases, the eye is situated near the extreme anterior end of the ganglion.

4. In this case (Fig. 23) the fusion of the auricles at the front of the head is even more complete than in Case 3, only the bases of the sensory areas being separated. The eye is median and apparently single and the pigment spot is of rather large size. Anterior to the eye the ganglionic mass breaks up almost imme-



FIGS. 23–27.

diately into two nerves passing to the front of the head (Fig. 24). In Fig. 25 it becomes evident that the apparently single eye is actually double. One of the pigment cups lies slightly anterior and ventral to the other and somewhat to the left of it. The opening of the more posterior and dorsal cup is seen in Fig. 25, while the other pigment cup appears here as a complete circle. Both open antero-ventrally and toward the right. The ganglionic mass is not divided into right and left halves and the optic nerves arise from its median region. Both eyes are far below the dorsal surface of the body and the more ventral one is imbedded in the ganglion.

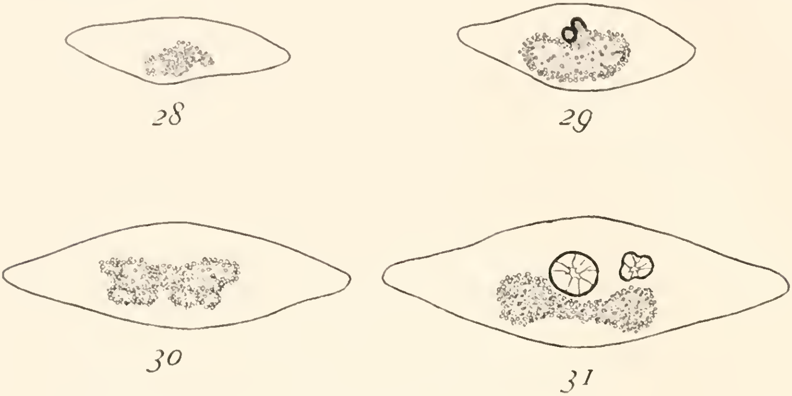
The double nature of the eye is not apparent in the living animal since the two pigment cups lie so close together and one is almost ventral to the other.

Posterior to the level of the eyes the ganglion is abnormal in form to about the posterior end of the regenerated region. Fig. 26, a section slightly anterior to the boundary between new and old tissue, shows that in general form and arrangement of the fiber tracts the ganglion at this level resembles that of Case 1 (Fig. 13). A little farther posteriorly, in the old tissue, it divides into right and left portions and gives rise to two nerve cords of the usual form, but somewhat nearer together in their anterior region than in normal forms.

5. This case is like Case 4 in external appearance (Fig. 23) and also in the number and arrangement of the eyes, but some differences in the structure of the nervous system exist. Fig. 28 shows the level of origin of nerves to the front of the head, a level slightly anterior to the eyes. In Fig. 29 the eyes are seen to be slightly larger than in the preceding case, but otherwise similar to it. Both open antero-ventrally and toward the right and one lies to the left of, ventral and slightly anterior to the other. The ganglionic mass is single, but larger than in Case 4 at this level (Figs. 29 and 25) and the optic nerves arise from it somewhat to the right of the middle.

Posterior to the eyes the ganglion soon shows distinct right and left halves but these are abnormal in shape and each is broken up into a number of more or less distinct fiber tracts, which, however, are apparently symmetrically arranged in the right and left halves (Fig. 30). This peculiar arrangement continues to about the posterior end of the regenerated region and then changes into the form shown in Fig. 31: posterior to this the two ventral cords appear in the usual form.

These five cases give some idea of the variations in structure of the eyes and the nervous system in the teratomorphic forms. A more extended investigation of these forms will undoubtedly show other variations in structure and examination of anophthalmic and headless forms will add still further data of interest.



Figs. 28–31.

V. Discussion.

In all of the cases described, both the teratophthalmic and the teratomorphic forms, the most conspicuous difference in the nervous system as compared with the normal forms is the more or less complete fusion in the median line of the two portions of the ganglionic mass, or more correctly, their incomplete separation.

It is evident that to some extent the condition of the eyes or eye is an index of the condition of the nervous system. The parallelism is, however, not complete: in Case 1 (Figs. 10–15), for example, the nervous system is much more abnormal than in Cases 2 (Figs. 16–18) and 3 (Figs. 19–22), though all three possess a single median eye.

Moreover, the degree of fusion of the auricles does not correspond exactly to the degree of fusion of the ganglia in all cases.

In Case 1 where the auricles are a considerable distance apart (Fig. 10) the fusion of the ganglia (Figs. 11–15) is more complete and their structure is more abnormal than in Cases 2 (Figs. 16–18) and 3 (Figs. 19–22), where the auricles are nearer together. In Cases 4 and 5, where two eyes develop close together, far from the surface and in abnormal relations to each other and where the auricles are partially fused, the ganglionic region is highly abnormal.

When, however, we compare the teratophthalmic with the teratomorphic forms it is evident that a general parallelism between the external features of the head and the condition of the nervous system does exist. So far as the observations go at present, the nervous system is always more abnormal in the teratomorphic forms than in the teratophthalmic forms with partially fused eyes.

As regards the eyes themselves certain points are of interest. In the normal and partially fused eyes the pigment cups open laterally, while in the teratomorphic forms they open anteriorly or antero-ventrally. Moreover, the eyes are usually farther from the dorsal surface of the head in the teratomorphic forms than in others.

The single eye of the teratomorphic head may be connected with both sides of the ganglionic mass (Fig. 18) or with only one (Fig. 21) and in case two optic cups arise in the teratomorphic head (Figs. 25 and 29) they may both be connected with the same part of the ganglion.

The position of the eyes or eye on the ganglion may differ more or less widely in the normal and abnormal forms. In the normal animal (Figs. 1–5) the eyes lie dorsal to the posterior region of the ganglion, which divides a short distance behind them into the two nerve cords. In the teratophthalmic forms with partially fused eyes a considerable portion of the ganglion lies anterior to the eyes (Fig. 7) but the right and left sides of the nervous system remain united farther posteriorly than in the normal (Figs. 5 and 9). In the teratomorphic forms the eye lies dorsal to the extreme anterior portion of the ganglion and the two cords do not become separated for a considerable distance posterior to it.

In the development of the abnormal forms the eye undoubtedly arises in connection with the central nervous system as it does in the normal animals. The position and number of the eyes must be determined primarily by the condition of the nervous system, though other factors may play some part. Apparently the median regions of the nervous system are more or less reduced or fail to develop in the abnormal forms and the lateral regions consequently lie nearer together so that the eyes appear near or in the median line. The approximation and fusion of the auricles is also evidently due to the reduction or absence of the median region of the head and this condition is undoubtedly closely connected in one way or another with the condition of the ganglia. There can be no doubt that the condition of the nervous system is the most important factor in determining the characteristic features of the teratophthalmic and teratomorphic heads.

One of the most interesting points in connection with the whole series of forms is the fact that in the teratomorphic forms the whole length of the regenerated nervous system is abnormal (Figs. 13 and 14, Fig. 26, Fig. 30). Not until the level of the old tissue is reached do the two cords appear in their usual relations and even there they are commonly nearer together than in normal animals (Figs. 15, 22, 27, 31). This fact suggests that the development of the regenerated portion of the nervous system is in large measure independent of the already existing portion. If the development took place in the anterior direction from the cut ends of the nerve cords in the old tissue, it is difficult to understand how such structures as those shown in Figs. 13 and 14, 26 and 30 could arise near the old tissue. But if the development of the regenerated part takes place independently of the old part, the continuation of the abnormal structure back to the level of the preëxisting portion constitutes a less difficult problem.

According to recently published work of the senior author (Child, '11*d*) the formation of a new whole from a headless piece of *Planaria* consists essentially in the formation first of all of a new head region which then reorganizes the parts posterior to it through correlation. The structure of the regenerated portion of the nervous system in the teratomorphic forms certainly offers more support to this conclusion than to that view which maintains that the regenerating nervous system grows out anteriorly from the cut ends of the old nerve cords. Apparently in these cases a new central nervous system develops and is abnormal from the beginning, but as its differentiation extends posteriorly it meets the old nerve cords and unites with them. In this way it is easy to account for the relatively sudden change in the structure of the nervous system as we follow it posteriorly from the new into the old tissue in some of the teratomorphic forms (Figs. 14 and 15, 26 and 27, 30 and 31). But even the anterior regions of the old nerve cords undergo reorganization to a greater or less extent under the influence of the new region anterior to them. In Figs. 15, 27 and 31 they are nearer together and connected by larger commissures than they were originally when they formed a part of the posterior region of the first zoöid.

The structure of the nervous system in the posterior part of the regenerated region in such cases as Fig. 14 and Fig. 30 suggests a breaking up into separate nerves, but posterior to the levels of these sections where the developing portion meets the old cords there is a return to something approaching normal structure. It is possible that if the old cords were not present in such cases the new nervous system would extend posteriorly as a considerable number of separated cords or nerves instead of in the form characteristic of normal animals.

It was pointed out in Section I. that the teratophthalmic and teratomorphic forms can be produced experimentally by decreasing the rate of the dynamic processes in the piece below a certain rate necessary for the production of normal animals which is itself not constant but dependent upon various conditions. These abnormal forms then represent planarian morphogenesis corresponding to certain rates of reaction below the "normal" rate for the existing conditions. The fact that changes which are primarily quantitative give rise to such differences in structure as those recorded is important. As the rate of reaction decreases we see certain parts, *e. g.*, the preocular region of the head, decreasing in relative size and finally disappearing and in the nervous system the bilateral structure of the ganglia becomes less and less distinct in consequence of the reduction and disappearance of the median regions. Apparently we are justified in concluding that the reduction and disappearance of certain parts as the rate of reaction decreases is due one of two alternatives: first, the reduced or absent part may represent a relatively low rate of reaction in the normal animal and under the experimental conditions the rate of the reaction which is essential for its formation approaches or falls below what may be called the morphogenic threshold, *i. e.*, it does not produce the characteristic morphological effect. Second, a part may be reduced or disappear under conditions which decrease the rate of reaction, not because the reaction concerned in its formation is directly affected by the experimental conditions, but because its formation depends upon correlation with some other part which is thus affected. It is probable, for example, that the condition of the central nervous system in the abnormal forms is largely, at least in the cephalic ganglia, a direct effect of the experimental conditions, while the position, number and presence or absence of the eyes and the degree of development of the preocular region are to a considerable extent correlative effects.

But however we may account for the results it is a demonstrated fact that the reduction and disappearance of parts of so "essential" an organ as the central nervous system can be brought about experimentally by quantitative changes in external or internal conditions. No absence of chromosomes or determinants and no germinal variation is necessary for the production of these abnormal forms, but only a decrease in the rate of the dynamic processes in the piece, together with the necessary correlative effects of such a decrease.

It is impossible to leave the subject without some reference to the "cyclopean" fish embryos which Stockard ('07, '09, '10) has recently produced by means of magnesium chloride and alcohol. The resemblance between these forms and the teratophthalmic and teratomorphic forms of *Planaria* is striking. In both cases organs which are normally bilaterally symmetrical in position show various degrees of approach and in the extreme types a single median organ develops in place of the two. Anophthalmic forms also occur in *Planaria* and under extreme experimental conditions completely headless forms also appear.

Moreover, in *Planaria* the auricles, like the eyes, show various degrees of approximation and fusion and in the present paper it has been shown that similar conditions appear in the cephalic ganglia themselves. In *Planaria* these monstrous forms can be produced, not merely by anesthetics, but by a variety of conditions the essential effect of which is a decrease in the rate of the reactions in the living system. It seems probable that Stockard's cyclopean embryos and the other intermediate forms between these and the normal animals are the result of a decrease in the rate of reaction rather than of any specific anesthetic effect of either magnesium salts or alcohol. Moreover, the double or partially double heads which Stockard obtained in some cases are also readily accounted for on a quantitative basis: a decreased rate of reaction means decreased correlation and this condition favors physiological isolation of parts and reproduction, as the senior author of the present paper has shown elsewhere (Child, '11*f*).

The problem of the relation between morphogenesis and the rate of reaction in organisms is one of great importance, but it has received comparatively little attention. Current hypotheses of development and inheritance scarcely consider the possibility of altering the characteristic morphological features of the organism by changes in the rate of reaction, but of the fact there can be no doubt.

VI. SUMMARY.

1. The teratophthalmic and teratomorphic forms of *Planaria dorotocephala* can be produced experimentally by decreasing the rate of the dynamic processes in the isolated pieces below a certain variable level which is necessary for the production of normal forms.

2. In these forms the cephalic region of the nervous system differs more or less widely from that of normal animals. The two ganglionic masses are always less completely separated than in the normal animals and often only a single ganglion develops. In the teratomorphic forms the ganglia are more abnormal than in the teratophalmic forms.

3. In the normal animals the cephalic ganglia extend a considerable distance anterior to the eyes and the two separate

nerve cords arise near the level of the auricles. In the teratophthalmic forms with partially fused eyes the eyes lie nearer the anterior end of the ganglia and the right and left portions are not separated at the level of the auricles. The eyes of the teratomorphic forms are situated at the extreme anterior end of the ganglionic mass.

4. The abnormal structure of the nervous system in the teratophthalmic and teratomorphic forms continues posteriorly through the regenerated anterior end to the level of the old tissue and even the nerve cords in the old tissue may be more or less different from the normal. In some teratomorphic forms the regenerated nervous tissue apparently begins to break up into separate nerves a short distance posterior to the eyes, but resumes the form of two nerve cords in the old tissue.

5. In the normal animal the optic pigment cups open laterally and the same is true for the teratophthalmic forms with partially fused eyes. In the teratomorphic forms the cup opens anteriorly or antero-ventrally and in some cases more or less to one side. The single median eye may be connected by two nerves with right or left portions of the ganglionic mass, or by a single nerve with either one, or the optic nerve may arise from the median region of the ganglion. The eyes are also farther from the dorsal surface in the teratomorphic than in the normal forms and are sometimes more or less completely imbedded in the ganglionic mass.

Hull Zoölogical Laboratory,
University of Chicago,
October, 1911.

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'11e Studies on the Dynamics, etc. III. The Formation of New Zoöids in Planaria and other Forms, Journ. Exp. Zoöl., Vol. XI., No. 3. 1911.

'11f Die physiologische Isolation von Teilen des Organismus. Vortr. u. Aufs. ü. Entwickelungsmech., H. XI., 1911.

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'07 The Artificial Production of a Single Median Cyclopean Eye in the Fish Embryo by Means of Sea Water Solutions of Magnesium Chloride. Arch. f. Entwickelungsmech., Bd. XXIII., H. 2, 1907.

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EVIDENCE ON THE ADAPTATION OF PARAMÆCIA TO DIFFERENT ENVIRONMENTS.

LORANDE LOSS WOODRUFF.

The fact being established that my pedigree culture of *Paramæcium aurelia* (I.) undoubtedly has unlimited power of reproduction without conjugation or artificial stimulation,[1] a culture of *Paramæcium caudatum* was started for comparison, in order to determine if this animal would show throughout its life history characteristics of specific value and also to determine if it would continue to live and reproduce indefinitely without conjugation or artificial stimulation.

The results with this culture led me to conclude, as did Jennings and Hargitt,[2] that *caudatum* is a distinct species. This point I have discussed in a previous paper.[3] The results in regard to the second point are briefly presented at this time.

The pedigree culture of *Paramæcium caudatum* (X.) was started on May 14, 1910, and has been continued under observation to the present time, December 1, 1911. The methods employed have already been described in detail in earlier papers on pedigree cultures of Infusoria. It is only necessary to state here that the culture was begun by placing a large "wild" individual on a depression slide in about five drops of culture medium. When this individual had divided twice, producing four animals, each of these was placed on a separate slide, forming the four lines of the culture. Thereafter (until June 1, 1911) a single cell from each of the lines was isolated daily in fresh culture medium and the number of divisions during the previous twenty-four hours was recorded.

In regard to the culture of *Paramæcium aurelia* (I.), which served as a control and for comparison with the *P. caudatum* culture, there are no results to be recorded which are not in entire agreement with these already published. The culture has kept on the even tenure of its way and is now, after over four and one half years of daily observation, at the 2,705th generation, and in every way in as normal morphological and physiological condition as at the start. Given a favorable environment, this race clearly has unlimited power of reproduction without conjugation or artificial stimulation.

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[1] L. L. Woodruff: "Two Thousand Generations of *Paramæcium*." *Archiv für Protistenkunde*, Bd. 21, 3, 1911.

[2] H. S. Jennings and G. T. Hargitt: "Characteristics of the Diverse Races of *Paramæcium*." *Journ. Morph.*, Vol. 21, no. 4, 1910.

[3] L. L. Woodruff: "*Paramæcium aurelia* and *Paramæcium caudatum*." *Journ. Morph.*, Vol. 22, no. 2, 1911.

The pedigree culture of *Paramæcium caudatum* (X.), which was subjected from the start to the 500th generations (twelve and one half months) to identically the same treatment and culture medium as the *P. aurelia* culture, showed during the first 350 generations (eight months) essentially the same rate of reproduction as the *aurelia* culture. However, an examination of the data (cf. Figs. 1 and 2) shows that a slow decline in division rate set in at the start which finally resulted in a race of cells possessing many of the morphological and physiological characteristics described by Calkins[1] in his careful study of pure lines of this species of *Paramæcium*. After about the 450th generation it became increasingly difficult to keep the animals alive on the slides in the culture medium which was supplied fresh daily. However, the cells left over from the daily isolations, which were allowed to accumulate in the old culture liquid, appeared healthy and continued to reproduce slowly. If these were transferred again to fresh medium they would divide a few times and then die. Finally they would not live twenty-four hours in the fresh medium.

By substituting from the "stock" in this way, the direct lines of the culture were kept replenished for nearly three months; but finally it was evident that it was impossible to continue the culture by this method, so that the exact number of generations could be determined, and accordingly, at the 500th generation, the method was abandoned, and the animals were thereafter carried in small flasks of old infusions, *i. e.*, they were bred in a comparatively large volume of the same type of medium to which

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[1] G. N. Calkins: "The Life Cycle of *Paramæcium caudatum*." *Archiv für Entwickelungsmechanik der Organismen*, Bd. 15, 1. 1902. "Death of the A Series of *Paramæcium caudatum*. Conclusions." *Journ. Exper. Zoöl.*, Vol. 1, no. 3, 1904.

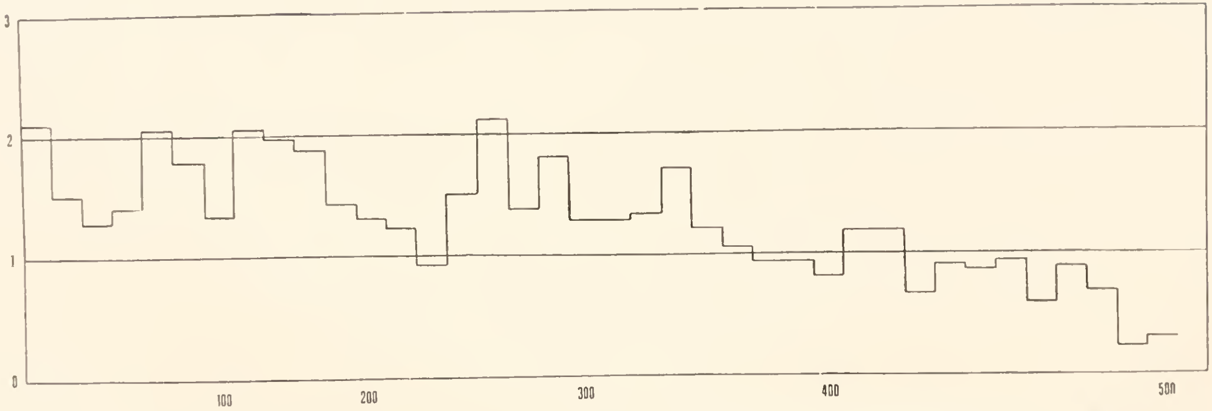

FIG. 1. *Paramæcium caudatum* (Culture X.). Graph of the rate of reproduction for the first 500 generations (May 14, 1910 to June 1, 1911). See text.

The average rate of division of the four lines of the culture is again averaged for *ten-day* periods. The figures 100, 200, etc., represent generations and are placed below the ten-day periods in which they were attained.

they had been previously subjected—but a medium which was from several days to several weeks old.

Under these conditions this culture of *P. caudatum* now flourishes, and it is continued by isolating a few cells every few weeks and inoculating with them another small flask of old infusion. Under these conditions it is impossible, of course, to determine with accuracy the rate of division or the number of generations attained to date, but the organisms are apparently in a normal physiological condition. However, it is still impossible to keep them alive on slides in the regulation five drops of fresh culture medium, which has proved so highly favorable for the *aurelia* culture.

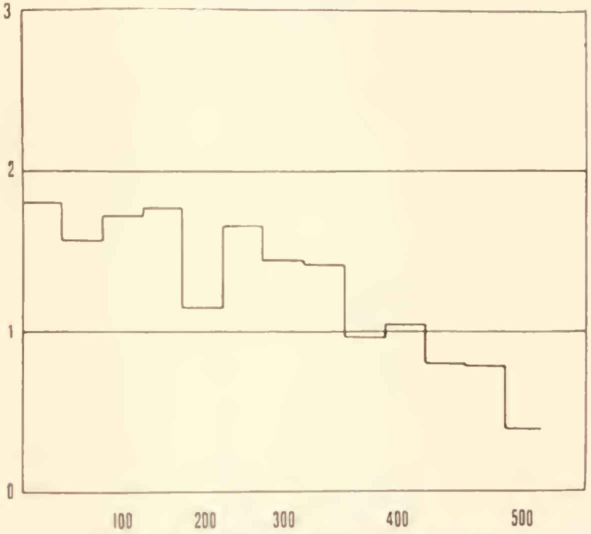

FIG. 2. *Paramæcium caudatum* Culture X. Graph of the rate of reproduction for the first 500 generations (May 14, 1910, to June 1, 1911). See text.

The average rate of division of the four lines of the culture is again averaged for periods of *one month*. The figures 100, 200, etc., represent generations and are placed below the months in which they were attained.

Now the question arises: Have the cells conjugated in the larger volume of medium and so been "rejuvenated." Since I have been unable to isolate the animals each day, I cannot *prove* that conjugation has not occurred, for it is possible that one or a few pairs have conjugated unobserved and have given rise to the present generations. The only way to prove that conjugation has not occurred is to make the conditions such that it is an impossibility for it to occur, *i. e.*, by *daily isolations and record of generations*. Since the physiological condition of this pedigree culture prohibited this after the 500th generation, I have adopted the method employed by many investigators of problems of this nature and allowed the Infusoria to accumulate in considerable numbers. I have, however, in order to increase the accuracy of the method, confined the cells in as small a volume of old infusion as possible and have examined the flasks at frequent intervals for signs of conjugation. I have never seen a single pair of conjugants in all the multitude of cells which I have examined, and it seems *highly improbable* that conjugation has occurred. It should be emphasized that, if conjugation has taken place, it has not so altered the physiological condition of the cells that they will live under the slide method of culture.

This culture, then, is apparently in as healthy a condition as at the beginning of the work, but it has become so modified that the animals are unable to exist in small volumes of fresh infusions. This is a decidedly interesting result in the light of the work of other investigators on *Paramœcium caudatum*, since it shows that a race of cells may exhibit all the signs of "senile degeneration" at the end of a typical "cycle" of generations, and still may appear healthy and exhibit a normal rate of reproduction when put under other conditions which approximate what is probably the usual environment of wild paramæcia.

In other words, this culture of *P. caudatum* substantiates the conclusion of Calkins that, under the conditions of his experiments, this organism may pass through a "cycle" which finally terminates in death; but it further shows that this "cycle" is probably an artificial one which is brought about by the subjection of the race to an environment which is not suitable for its prolonged existence. This culture also shows that pure lines of different species of *Paramœcium* (*aurelia* and *caudatum*) are adapted to different environmental conditions, in view of the fact that the race of *P. aurelia* has thrived indefinitely on the same culture medium which has proved increasingly unfavorable for the race of *P. caudatum*. It may be that this is actually a specific difference, but I believe that the fact that these two races belong to different species is merely an incident and that it will be found to be equally a variation of different pure races of the same species as the results of Jennings clearly indicate.[1]

Conclusions.

1. The discrepant results of various workers on the longevity of paramæcia is in all probability due to variations in the cultural demands of the races isolated for study.

2. It is probable that most, if not all, normal individuals have, under suitable environmental conditions, unlimited power of reproduction without conjugation or artificial stimulation.

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[1] Jennings and Hargitt: *loc. cit.*, p. 538. Jennings: *Amer. Naturalist*, Vol. 45, p. 83. 1911.

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BIOLOGICAL BULLETIN

OBSERVATIONS ON THE BEHAVIOR OF TUBICOLOUS ANNELIDS.

III.

CHAS. W. HARGITT.

In two earlier papers dealing with the general subject of the behavior of tube-dwelling annelids the writer endeavored to give in some detail an account of experiments and observations made upon several species of these worms available at Woods Hole, and incidentally made reference to a few observations upon one of the Naples species, *Protula protula*. During a recent occupancy of the Smithsonian table at the Naples laboratory occasion was taken to extend these observations to several other species, and to make as critical a comparison as might be practicable of the behavior of the latter with that of the Woods Hole species. It will be noted that in the present account less attention has been given to details of time reaction, various stimuli, etc., than before, and that behavior in relation to light has been emphasized. This was deemed the more important since it was upon these species that some of the earlier work concerned with animal heliotropism was done. As may be recalled, my work already mentioned ('06, '09), did not tend to confirm these views; and it was with a view to test them by a repetition of the experiments that I undertook to reëxamine the subject. In the following account will be found the results and conclusions which my observations have seemed to warrant.

The following are the species which have been used: *Protula protula*, *Hydroides pectinata* (*Serpula uncinata*), *Pomatoceras triqueter*, and *Spirographis spallanzanii*. The experiments were carried on from January 1 to April 15, a period of three and one half months. Particular pains were taken to vary the experiments in every practicable way, and under a range of conditions which would eliminate as fully as might be errors of inference based on limited experiments or faulty environmental conditions. Details on these points will be given in later sections of the paper.

PROTULA PROTULA.

This annelid is a very familiar element of the fauna of the Bay of Naples. Its large size, often 175 mm. in length by 5–8 mm. in diameter, its fantastically coiled tube, and the brilliant orange-red gills which are splendidly displayed during expansion conspire to make it a conspicuous object. The sensitiveness of the creature to differences of light intensity, such as that involved in the intervention of shadows, was one of the first aspects of behavior to engage my interest many years ago, some brief notice of which was made in my early paper ('06, pp. 311, 314). These observations I have verified again and again during the present series of experiments. Careful comparisons of many specimens in their reactions reveal the fact of marked individuality as expressed in the variability of behavior shown from day to day. It is not necessary to go into details concerning this point. What has been pointed out in the case of *Hydroides dianthus* ('09) is confirmed in the case of *Protula*. Certain specimens were especially sensitive and extremely active in response, while others would show the very opposite; and it was not unusual to find specimens which seemed totally indifferent to shadow stimuli. Again, specimens might prove quite sensitive at a given time and very indifferent at another. But let it be noted that some specimens seemed normally to be highly sensitive, while others seemed normally quite the opposite. Again, the retraction aspects of behavior, that is, the time a given specimen remained in the tube after a given contraction, was remarkably variable. In some cases the emergence was relatively prompt, while in others it was extremely slow. In this matter *Protula* differs materially from *Hydroides*, whose retraction periods are usually and normally very brief. *Protula* often remained retracted for indefinite periods, often for one or two hours at a time, in marked contrast to *Hydroides*.

Tubular Aspects.—The behavior of *Protula* as expressed in the form or aspects of the tubes is noteworthy. In my early paper was shown a figure which made this very graphic. No less than in the case of *Hydroides* the tubes of *Protula* show the record of erratic behavior in very striking manner. (Cf. '09, pp. 180–183.) During early life these tubes usually adhere very strongly and closely to the base of support; but in maturity they often incline more or less toward the vertical, though in a rather sinuous or spiral direction, or may even coil about each other and assume a downward aspect. This may be seen most strikingly in the large colonies of these creatures always present in the show aquaria of the laboratory, where may also be seen to best effect the marked variability as to tubular behavior. Something of this is graphically shown in Figs. 1 and 3, copied from photo-

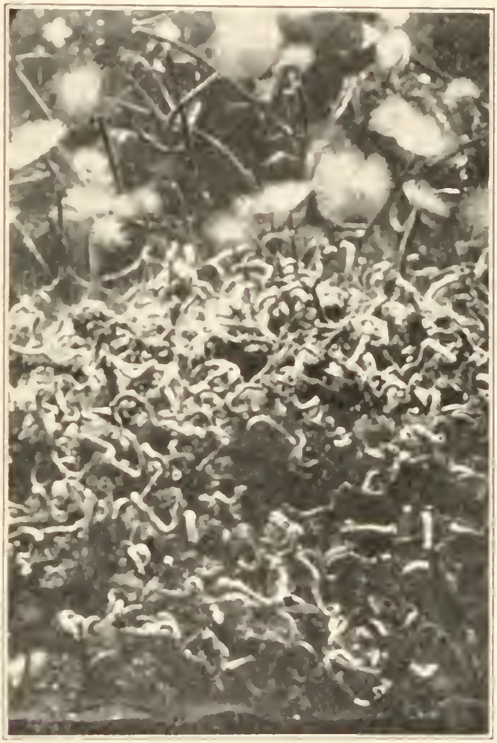


FIG. 1 shows a large colony of *Protula*, with *Spirographis* in the upper part. The promiscuous curving of the tubes is very marked.

graphs taken by Dr. Sobotta, by whose kind permission I am able to present them here. As will be seen, the aspects of the tubes and of the openings through which the creatures protruded their heads are so extremely diversified as to seem to be absolutely chaotic. If one may distinguish any tendency toward a given aspect of position, still the departures are so numerous as to render it almost certain that no single factor could have been determinative. As in the case of *Hydroides* ('09, p. 180, etc.), *Protula* has left in its tubes a convincing record of the erratic individuality of its behavior the significance of which is extremely important.

Autotomy.—In this connection may be considered a feature of behavior more or less unique, though not peculiar to *Protula*, since it has been noted in several cases of *Spirographis*; namely, that of autotomy, or the self-excision of certain organs of the body. This was first observed in the case of *Protula*. A specimen of this worm was among the first to come under my observation, having come to my table almost the first day in the laboratory. It had been placed in a small aquarium jar on the table for convenience of study. After finishing a given series of tests the specimen was usually returned to the large aquarium. On January 7 the specimen had been under observation and was left in the table jar which had a capacity of about four or five liters, while I went out to lunch. This could hardly have been more than an hour or so, but on return I observed what seemed strange—the detached portion of about half of the gill mass lying at the base of the tube. An examination of the gill failed to reveal any signs of disease or other abnormality. My first impression was that possibly the water had become "*bad*," yet other living things, such as copepods, showed no signs of discomfort. However the water was renewed several times during the afternoon and the specimen finally left on the table over night, as had been done several times before. The following morning upon examining the jar I found the other portion of the gill in the same detached condition, lying at the base of the tube, but the specimen was deeply retracted within the tube. After some time it came to the orifice and showed clearly that it was entirely devoid of gill elements. It was now transferred to the large aquarium and left to see whether regeneration would occur and if so at what rate. For a few days it frequently came to the orifice and extended the mantle edge over the margin of the tube and remained in that condition for some time. Later it again withdrew deeply into the tube and did not show itself for several days, indeed for some three weeks or more. Finally, on February 13 it was once more seen to protrude its head, but there was not the least sign of any regeneration. Its appearance now became more frequent and occasion was taken to test it by shadows, and to my surprise it was found to react as promptly as at the very first. These tests were made many times on subsequent days with the almost uniformly prompt and positive reaction, but with the variations observed at first, *i. e.*, sometimes less sharp, and then more so, and occasionally not at all.

Several interesting questions arise in this connection. First, as to the regenerative capacity of *Protula*. For nearly two months not a sign of regeneration was distinguishable. I had previously recorded ('06) the promptness with which *Hydroides* regenerated its gills, and similar records had also been made by Zeleny. Finally on March 14 I found undoubted evidence of regeneration, and this went forward apparently rather rapidly; for by April 10 the new gills had become quite conspicuous— nearly a fifth as large as the originals. It may be noted here that later I had also a similar autotomy by another specimen of *Protula* and by two specimens of *Spirographis*. In these cases there could not be doubt as to any condition of water inducing the autotomy, for the specimens continued to thrive, as did many others in the same tank. Regeneration was very prompt and rapid in these cases.

A second point is in relation to such correlation of function as would enable the creature during this long period of gill deprivation to maintain the normal respiratory activity. If respitation were restricted to the gills alone of course it must have perished. This experiment shows clearly that this function may be taken up by other organs of the body without serious inconvenience. But the gills are also concerned in the process of nutrition, acting as a medium for capture of prey. How might this function have been supplemented? It has been said that during this time the specimens remained rather continuously within the tubes. Did they depend wholly upon a reserve food supply?

It may not be possible to answer these queries fully, but of the correlation of the skin in the function of respiration there can be no serious doubt. In my earlier experiments on *Hydroides* ('06) it was found that when the gills were excised to test their relations to sensory reactions the creature did not seem to suffer any serious inconvenience as to respiration. So in the case of *Protula*, there was no evidence to the effect that respiration was not normal during the long period of gilless life. Bounhiol (1900) has reached similar conclusions from experiments on *Spirographis*. He finds that respiration takes place through both skin and gills, and that they supplement each other by compensatory interaction. He finds also that it is apparently easier for the gills to assume extra work than for the skin, and that in excretion of CO_2 the skin normally excretes about three fourths of the entire amount.

In the third place, there is the interesting query as to the sensory function. I have shown that for *Hydroides* light perception is almost exclusively a function of the gills. In *Protula* this is not so certain. Its behavior in this respect is less easily controlled, owing to the sulking disposition of the worm. But it is quite certain that autotomy did not result in entire inhibition of reaction to shadows and it may not be improbable that something of sensory compensation may obtain in this, as in the respiratory activity; or possibly this sensory function may be shared in part with some other head-organ, possibly the mantle margin, which in normal life is often extended over the orifice of the tube, hence in a position admirably adapted to such a function.

Concerning the entire matter of the significance of autotomy little can be said. Such phenomena, similar in many respects, are well known among other animal groups, though not common in any case, unless we may include phenomena of fission which is a very familiar feature in many annelids; but this seems to be a wholly different problem. That it is spontaneous, hence not attributable to the operation of gravity, contact, etc., seems very evident.

POMATOCERAS TRIQUETER.

This, with an undetermined species of *Serpula*, are tubicolous annelids which much resemble in general aspect of size, structure and behavior, *Hydroides dianthus*. Indeed in almost every particular they might have been substituted for the latter species without marked changes of results. In general habitat the two species are much alike and often found growing on the same substratum. *Pomatoceras* is rather larger, and its tubes are characterized by rather sharp triangularity with the dorsal angle forming a sharp crest along the entire tube. No attempt will be made to go into details as to matters of behavior, since as already suggested, they show the same reaction phenomena as those given in the accounts of *Hydroides dianthus*, and the growth aspects are quite as erratic. For the most part the tubes adhere closely to the substratum, and in some cases they adjust themselves with such nicety to grooves or similar depressions that one might guess they were under the control of some such stimulus as thigmotaxis or stereotropism. But when one comes to examine any considerable number of specimens he soon finds that in by far the larger number there is absolutely no such adjustment. The same is the case with *Hydroides*. Now and then a specimen may be found on a shell of *Pecten* in which there is a very fine illustration of such *appearance*, the creature having kept very closely and exactly in the radial grooves of the shell. But on the same shell may now and then be found another specimen which has grown directly across these grooves; and of course by far the larger number have absolutely no semblance of such response. The conclusion is therefore forced upon one that the operation of any such factor must be, if not wholly nil, yet of only incidental significance in behavior.

Again, in habitat one finds in the Mediterranean species the same wide range as in those of Woods Hole. I have dwelt upon this point with some emphasis in a former paper ('09, pp. 182–3), and need only refer to the matter in this connection by way of further emphasizing a point which I regard of considerable significance. The growth of these organisms indiscriminately on a large variety of substrata, rocks, shells—the latter both dead and living—nets, crabs, lobsters, etc., is itself of no small import as to the negative influence of such factors as light, gravity, etc., in relation to growth. This is further borne out by attention to the aspects of the several tubes which may comprise a given colony. In several such an actual count of the growth direction was made. On a stone which contained 37 living specimens I found that 12 had a general upward direction; 15 had just as definite a downward course; and 10 had a horizontal direction. Another colony growing on the inside of an iron cup about 6×10 cm., made up of eleven specimens, showed the following disposition as to direction: 4 upward, 5 directly down, and 2 horizontal. On the outside of the same cup were eighteen specimens disposed as follows: 8 upward, 7 downward, and 3 horizontal. These plainly go to confirm the conclusions already drawn, that in the matter of orientation one is utterly unable to discover the operation of any one or several factors which are in any sense determining.

HYDROIDES PECTINATA.

This species and the one described in the following section, *Spirogaphis spallanzanii*, were the ones used by Loeb in his well-known experiments at Naples many years since, the results of which, including also several species of hydroids, served as a basis for his far-reaching theory of animal heliotropism, especially as it relates to sessile animals. Naturally, therefore, more of details will be expected in'the following accounts than in the preceding, and I shall endeavor to make explicit and definite records both of methods and results.

Hydroides pectinata (*Serpula uncinata*) is one of the most common and abundant of the Naples annelids. Unlike *Spirographis*, it grows usually in immense colonies, aggregating hundreds or perhaps thousands of individuals. Fig. 2 will give a general idea as to their appearance in small colonies. The tubes of a given colony form a mass of more or less parallel aspect, the individuals apparently growing at approximately the same rate and in the same general direction. When one casually examines such a colony it would seem to afford a typical illustration of orientation due to some single constraining stimulus. But here again, as in the case cited above as to stereotropism, extended observation brings to light too many exceptions to any such rule, and compels further inquiry. Like other species of *Hydroides* this one secretes a calcareous tube, the shape of which depends upon the mode of growth of the individual constructing it. Hence in aspect, size, etc., these become permanent records of everyday behavior, whether this be mechanical, ecological, or physiological in its nature. Several colonies were put under observation early in January and were tested during a period of more than three months; to be more exact, from January 3 to April 12. They were tested as to the possible influence of both light and gravity. Loeb claims that in this species the reaction is quite prompt. "I noticed that in the course of the next day

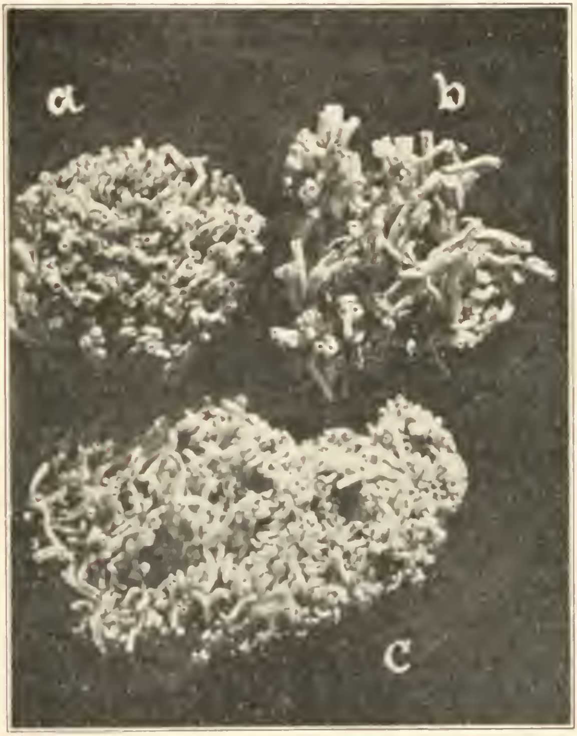


FIG. 2, *a*, *b*, *c*, shows three colonies of *Hydroides pectinata* which have been under light test for more than a month. As will be seen the curvings of the tubes are much as in Fig. 1.

the Serpulidæ, which like *Spirographis* presented only their gills to the light, bent them strongly upward" ('90, "Gen. Phys.," Part I., p. 102), and he continues, "within six weeks the entire block was covered with tubes which curved upward; not a single individual had continued to grow in the original direction," and presents a figure in illustration. There is apparent discrepancy between the latter and the statement "*not a single individual,* etc.," for in the figure about as many appear to "continue to grow in the original direction" as have "curved upward." My own experiments show a reasonable conformity to Loeb's *figure,* but the ratio of tubes indicating reaction is very much smaller. Figs. 2, *a*, *b*, *c*, are photograph reproductions, and may therefore be taken at their face value, and they certainly fail to show any such response as claimed above. For example, it was found by actual count of a colony comprising hundreds of tubes which had been under test for more than a month that only about twenty tubes had definite curves toward the light, a similar number had shown lateral curvatures, and a smaller number had curved downward; but the larger number "had continued to grow in the original direction." A smaller colony which had been under test for twenty-five days under particularly favorable light conditions showed a slightly larger response toward the light; but here also the number was relatively small. Another colony was placed in an aquarium which was covered on three sides and above with a black hood. After a test of nearly two months (January 23 to March 18), it was found by a careful estimate from counting that at most only about 20 per cent. showed any possible light reaction; while by far the greater number either continued to grow in the original direction or showed curvature laterally or downward. The colony was submitted to two others working at the laboratory, Dr. Butler, of University College, Dublin, and Dr. S. R. Williams, of Miami University, Ohio, both of whom made the per cent. of light reaction much lower than my own.

A very interesting and, I believe, significant feature of growth in this species came to light during the observations, namely, its very erratic, or discontinuous character. Some individuals showed a very prompt and rapid growth at first and later its cessation. In this process of rapid growth some show a bending while others do not. Again, some bend toward the light, others away from it, and still others continue in the original direction. The point of importance here is not the bending or curving, but simply the *tube-extension*. This extension is not, as I interpret it, an expression of *growth* at all, so far as the body mass of the animal is concerned. Seldom are aquarium conditions especially conducive to *physiological growth*. What then does such tube-extension mean? Isolated worms lying side by side, of essentially similar age, state of vigor, under identical conditions, show the most remarkable differences in relation to this matter of so-called growth. One may in the course of a week extend its tube 3–5 mm.; another shows not the slightest extension of its tube. One may extend its tube in the line of the body axis, *i. e.*, straight, the other show a sharp curvature from the first. There has been equal access to food, air, light. Why has not growth been the same in direction and amount? As a matter of fact it may be doubted whether there has been any appreciable growth. Indeed may not these erratic phenomena express just the opposite, namely, *lack of growth conditions*, or some other factors conducive to comfort? And if so then this erratic tube-extension is but an expression of such discomfort,—an expression of the efforts of the creature to seek better conditions, to reach out, as it were, in search of food, air, etc. Indeed, if my interpretation be correct, these curvings are but the natural expressions of efforts at food-getting or respiration—adjustments to those particular ends involved in survival or selection. In the light of this interpretation the real factors involved in these aspects of behavior are *intrinsic* and not *extrinsic*. The latter are conditional and passive; the former are individual, active, causative.

SPIROGRAPHIS SPALLANZNII.

This species differs most markedly from those already considered in that it possesses a very flexible tube, hence is capable of considerable range of movement within a region measured by the radius of its own length. It is a large species at maturity, averaging perhaps about 25 cm. in length, by about 1 cm. in diameter. In my experiments care was taken to have specimens of various sizes, and those actually used ranged from about 5 to 30 cm. in length. No less care was exercised as to conditions under which experiments were conducted. Three of the large aquaria supplied with running water were at my disposal during the period, and in addition two special experimental aquaria of smaller size, about 25 × 35 × 45 cm., also supplied with running

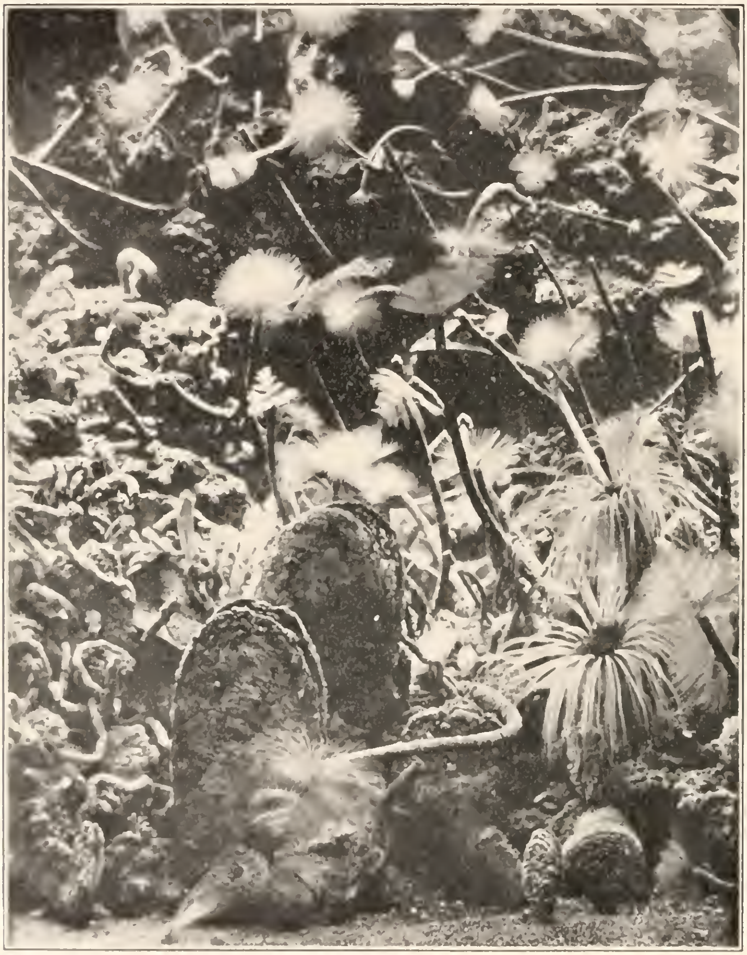


FIG. 3 is a portion of one of the large show-aquaria containing *Protula* and *Spirographis*. The varied aspect of the latter is quite marked, as will be seen by comparing specimens in various positions.

water, were employed for such experiments as called for a critical control of light, etc. Of the large aquaria two were in a room with north exposure, and hence with diffuse light, but always adequate for ordinary observation and experiment; while the other was in a room with direct south exposure, hence with sunlight of almost any degree of intensity, modified by shades or screens. The two smaller aquaria were in a special room, the light of which was under easy control, and the aquaria themselves easily adjusted to any desired condition, as to amount and direction of light, etc. A still further point is worthy of notice. At all times I had free access to the large exhibition aquaria, where large numbers of these specimens were living under conditions as nearly natural as the long experience and painstaking skill of those in charge have been able to devise. I shall have occasion to refer to this in another connection.

During the progress of the experiments some fifty specimens were available, and the general health and vigor may be inferred from the fact that in the three and one half months not a single specimen died or even showed signs of deterioration, except as a slight fading of the brilliance of coloration may have been indicative of such. Care was taken to supply food from time to time, almost daily, such as came in from plankton hauls which were supplied to the rooms quite regularly, and this may have contributed to the excellent conditions of health already alluded to.

Spirographis seems to take rather naturally to the aquarium environment and soon becomes quite at home so far as one may judge from appearance. Specimens require from two to several days firmly to attach themselves to the bottom or sides of the aquarium. This is accomplished by an adhesive secretion of the worm which is discharged through a small pore at the lower end of the tube. The time required for attachment may be varied by having the bottom of the aquarium covered with a layer of sand or by placing fragments of rock in contact with the base of the tubes. While in most cases the specimens attach themselves wherever they happen to be placed, which is fortunate in such experiments as those under consideration, still it not infrequently happens that a specimen will go through various translatory movements before finally settling down. It may be noted that these locomotor movements take place usually during the night. This I have demonstrated by carefully marking locations and noting subsequent changes. At no time have I found evidence of these movements during the day.

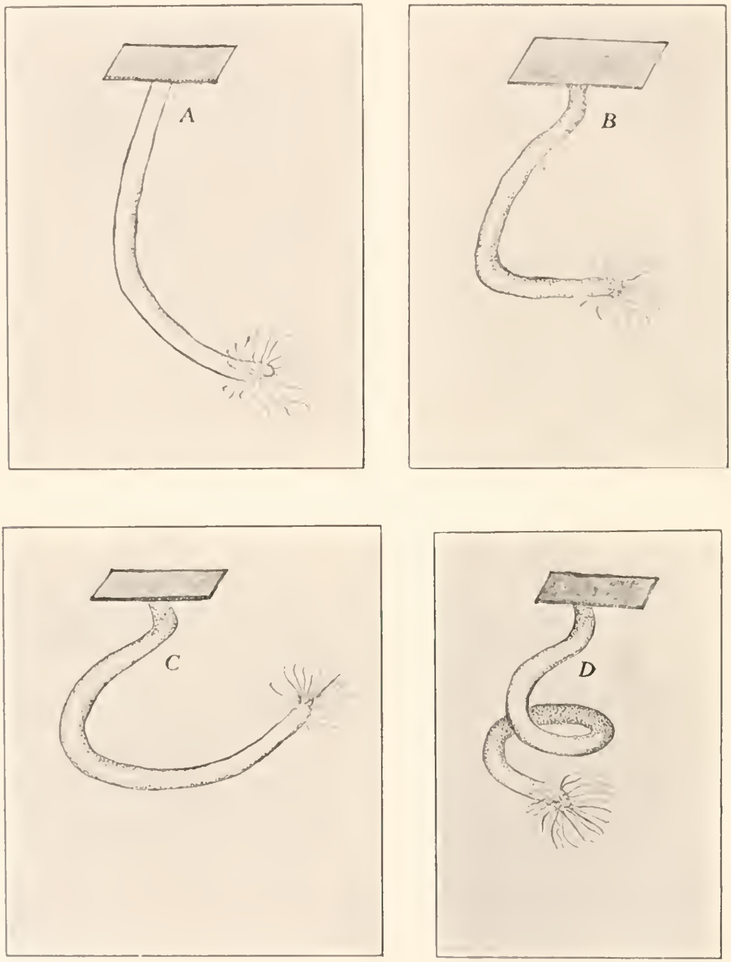
In general my experiments proceeded along the lines employed by Loeb ('90, *Arch. f. ges. Physiol.*, Bd. 47, p. 391), whose objective aim was to establish the essential identity of heliotropism in animals and plants, and his experiments were directed to that end. Incidentally it may be observed that he does not hesitate to claim "I think I have shown that the heliotropism of sessile animals is essentially identical with the heliotropism of sessile plants." And still later he asserts even more strongly, "It was possible to show that heliotropism of animals agree in every point with that of plants" ("Comp. Phys. of Brain," 1900, p. 181). It may be doubted whether, in the light of present knowledge, this would be seriously maintained. I shall not discuss the matter here further than to say that my own experiments were undertaken with a very different aim, namely, that of ascertaining the questions of fact,—*Are these organisms heliotropic?* and further, *Do they exemplify*, or *conform* to the *mechanical concept* of *behavior?*

In the following account I shall present the matter under some three distinct series. First, those experiments made in the aquaria located in a north room; second, those conducted in the smaller experimental aquaria; and third, those conducted in the large aquarium located in a room with exposure to direct sunlight.

The first series began on January 6 with some six specimens. To these additions were made from day to day, till on the 13th I had twenty, which had been variously distributed in the two large aquaria, some with the heads directed away from the windows, others directed at right angles, and still others facing the windows. The aquaria were of about the same size, probably 1.5 meters in length, by about 40 cm. in depth and width; the one with its end toward the window, the other with its side toward the light. It was some time after specimens had become attached before any sign of orientation was discernible. In the aquarium (No. 1) with the end directed toward the light there were twelve specimens, in the other eight. The twelve had been distributed so that three should face toward each of the sides of the tank; *i. e.*, three with heads directed toward light, three away from light, and six at right angles, three facing each side. On January 26 all specimens were attached except one, which for some reason, perhaps injury, remained free during the entire course of the experiment, hence may be disregarded. At this date the following is the record of orientation. The three facing the light continued in that position, one of which had assumed a nearly erect attitude; the other two had barely elevated the head to a degree sufficient to allow the gills to clear the bottom of the tank when expanded. Four specimens now face the wall, and all with barely sufficient up-bending to free the gills from the bottom. The laterally directed specimens continued as at first, except that one had made a distinct upcurve, the head elevated to an angle of about 35 degrees.

On February 6 the record of this tank is as follows: Of specimens facing light two are curved upward, one nearly vertical, perhaps 70°, the other about 45°, while the third remains as before, and this in spite of the fact that direct light is intercepted by a tufaceous mass bearing tubes of *Protula*, etc. The four specimens facing the wall have made considerable change. One had rotated through an arc of about 100 degrees, now facing the side, and with head elevated about 35 degrees. Another has also rotated to nearly right angles and curved upward 50 degrees. The other two continue unchanged. The specimens laterally disposed continue essentially as before, except an upcurve of from 30 to 40 degrees. The record for this aquarium on February 25 is as follows: Five specimens are now facing the wall, three continue to face the light, while the others continue essentially as before.

The following records of the behavior of the other aquarium, which may be called number two, are interesting. In this were placed eight specimens, two of which were suspended head downward, and in this position they attach themselves and continued for many weeks. The others were located with heads predominantly toward the wall, *i. e.*, away from source of light, only one facing light. In this tank but little sign of light reaction was distinguishable. The specimen originally facing the light later curved to the wall and remained in that position during the entire time, while one of the specimens placed facing the wall later curved toward the light side.



Figs. *A*, *B*, *C*, *D* illustrate certain aspects of a specimen which was suspended head downward. At *A* is shown the first indication of change of position; a further change is shown at *B*; this curvature has reached its limit in that form at *C*, and continued thus for several days, oscillating somewhat from side to side, but with no evidence of reaction to light. At *D* the sickle shape is converted into the loose spiral, which likewise continued for some days essentially as shown in diagram; as in the others, there was shifting and change but with no relation to light.

The most interest attaching to this experiment is the behavior of the specimens suspended. For several days both remained hanging downward. Finally one began to curve, and directly by a graduated process assumed the aspects shown in the diagram figures *A*, *B*, *C* and *D*. In the entire course of the experiment there was not the slightest indication of light response, nor indeed was there more of a geotropic character. The final attitude was that indicated in *D*.

The other suspended specimen attached itself to the side of the overflow tube, and has continued head down, without appreciable change of aspect, the tube remaining almost perfectly straight from first to last. Both specimens seemed equally at ease, both equally active; but the one passed through the series of tubular contortions, the other remained absolutely indifferent. Incidentally it may be remarked that specimens are often found in nature attached to the under surface of bottom of boats or other substrata, much like barnacles or other sessile organisms; and hence it must be admitted that there is nothing especially unusual or unnatural in such an attitude. That the behavior of the one differed from that of the other is not more strange than that differences likewise appear between others.

Special Aquaria. The second series of experiments were conducted in two special aquaria, mentioned above, and were prompted by two considerations First, the apparently negative character of the experiments began and carried forward in the large aquaria. It had seemed as if one should have more prompt and convincing results than appeared in the account just given. Secondly, it was desirable to have aquaria of a size and adjustment which made possible ready and effective control at all times, with such variation of tests as seemed desirable. Hence these smaller aquaria already described. They were set in a room whose light and temperature were under easy control, and were themselves of a size which enabled one to shift the position at any time it might seem desirable. It occurred to me that possibly the fact that in the first series the light had been diffuse rather than direct might have resulted in the somewhat negative behavior already noted. Again, it seemed desirable to be able to control both the direction and intensity of the light. Accordingly the special aquaria were made use of, and the following account is based entirely upon the behavior under the new conditions. Two were used for the definite purpose of making of one a control of the other. That is, given identical conditions of temperature, food, etc., will the mere difference of direction or intensity of light show itself in such measure as to warrant conclusions?

This series was begun on January 15 with twelve specimens, eight being placed in the experimental tank, and four in the control tank. The bottoms of the aquaria were covered by a layer of rather coarse, black sand to facilitate attachment, and at the same time to render any access of light from the bottom impossible. The test tank was covered on three sides and the top with an opaque hood, painted black on the inside and so adjusted as to render inspection easy without disturbing the specimens. In this tank the eight specimens were placed with heads facing away from the source of light. Similar disposition was made of the four specimens of the control tank. In about three days the specimens had apparently attached themselves, and on January 19, four days after beginning, one specimen began an upward curve. On the 21st several had shown such reaction and by the 25th several had curved upward to from 25 to 50 degrees. In the control tank similar responses began to appear.

On January 25, ten days after beginning, the record is as follows: Of the eight specimens two have curved toward the light, two are nearly vertical, two face toward the side, while two remain as planted. Essentially the same condition obtains in the control tank. One faces the window, one nearly vertical, and two as originally located.

At the end of four weeks, February 11, three show apparent light reaction, two are nearly vertical, two remain facing away from light, and one shows an indifferent curve laterally. The positions in the control tank remain as before. Repeating Loeb's experiment at this point, I now rotated the aquaria through 180 degrees, so that everything was changed directly about. Conditions went on as before, the test tank receiving light exclusively from one end, the control receiving diffuse light from the room as well as the direct light from the window. On February 25, or fifteen days after the aquaria had been rotated, the conditions are as follows: Five specimens now face the light, while three face the opposite direction. But of the five now facing the light three were so placed in the readjustment made by the rotating, or reversing of the tank, so that only two have actually shown a possible light reaction. The three specimens which had been turned away from the window by this reversal had not shown the slightest response.

At this time the aquaria were again reversed, so that they came back to the original positions. It should be noted that in the control tank there had been no change induced by the reversal of the position, the specimens all remaining as before.

Another aspect of behavior may be stated in this connection, namely, an actual downward curve of several specimens. It was on first notice thought that possibly this might be due to the incurrent water, which happened to be in the region of one such case. However, it was later observed that other specimens quite remote showed the same thing, and on comparing similar conditions in the exhibition aquaria it was found to have its counterpart there. Hence it may be regarded as only another expression of the individuality of behavior which is more or less evident under all conditions.

The experiments with these special aquaria were continued to March 25, having thus been under operation for about ten weeks (January 13 to March 25), and have been in the present position for exactly one month. During this time there have been incidental shiftings on the part of various specimens, a bending this way or that from time to time, but only to be reversed later, or counterbalanced by opposite reactions of adjacent specimens. These have been noted from time to time during the course of all the experiments, and are not to be considered as orienting reactions, but rather expressions of the individuality of behavior characteristic, as I believe, of all grades of animal behavior. They correspond to what Jennings has designated as *trial reactions;* and in the present instances probably relate to food-seeking or respiration. These statements refer directly to conditions in the darkened aquarium; but they are quite as applicable to those of the control aquarium, and indeed, the behavior of the specimens in this, while differing in various details, have shown a striking similarity to that of specimens in the former, as well as that of the first series in the large aquaria. As remarked in the outset, the entire series of experiments have involved no appreciable deterioration of the health or vigor of the specimens. As an evidence of this may be mentioned the fact that one very young specimen among those used in the control tank showed an apparently continuous growth, having nearly doubled its original size. The growth in this case seems to have been real and normal, and not *apparent* as was the case with *Hydroides*, mentioned in a previous section.

Third Series.—Early in March it was found desirable to change rooms in the laboratory, and I came into possession of one admirably adapted to light experiments. Advantage was taken of this circumstance to continue the experiments with *Spirographis* under light conditions which were exceptionally good. In the room were two large aquaria, one of which I devoted exclusively to this experiment. The aquarium was arranged with its side facing the window and at a distance of about two meters. By covering the back, ends and top of the aquarium with a black opaque screen, and with windows also provided with adjustable shades, one was able to directly control the light conditions at will, as to source, directness and intensity. The experiment was begun with eight specimens, all of which were placed with heads facing away from the light, and two others suspended head down by attaching them to sides of the overflow pipe, as in the similar experiment in series I. Other specimens were added a few days later making a total of twenty comprising the experiments. As before some two to four days were required for specimens to become attached to the aquarium. In the present case to insure prompt and precise location several were secured to a given place by putting over the terminal base of the tubes a small weight, such as a shell or rock fragment. As before the first indication of reaction was the usual upward curve of the oral end of the tubes, enabling the creature to freely expand the gills. This reaction has little, if any, relation to orientation movements, as it occurs usually in all cases and under almost all conditions, whether in light or darkness.

On March 25, ten days after the specimens were installed, only one had assumed a nearly vertical aspect. Others showed various phases of orientation, from ten to twenty, or thirty, or fifty degrees of elevation above the bottom.

On April 1, the following is the record. Four specimens with gills directed more or less toward the light; two with a vertical attitude; three oriented at right angles to direction of light, and facing darkest end of tank; nine remain oriented in original position, *i. e.*, facing away from light. The two suspended specimens behave almost exactly as in the previous case; that is, one perfectly unchanged and the other curved away from the pipe. Thus after nearly two weeks half of the entire lot remain absolutely unchanged; of the others only five show any very clear reaction to possible light stimulus. The experiments continued under daily observation until April 12, a period of one month, with a final record as follows: Four specimens show a distinct curvature toward the light; nine show just as distinct inclination away from the light, in other words remain as originally fixed except the slight curvature upward; two are almost vertical; the other three occupy positions at right angles to the line of light.

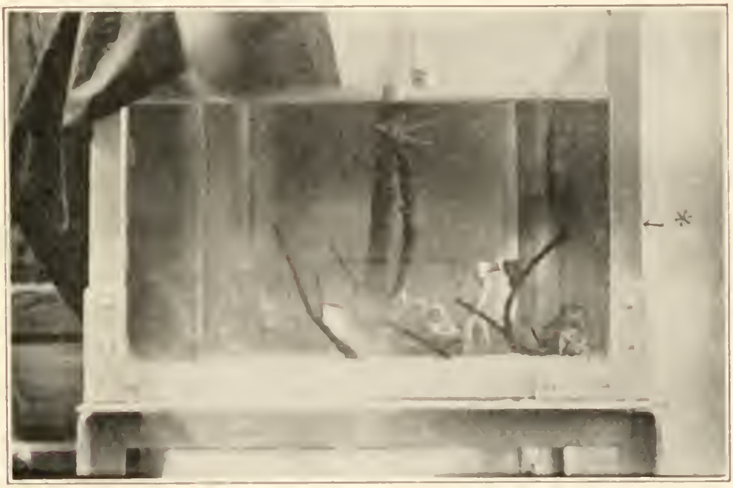


FIG. 4 is an end view of an experimental aquarium, the light coming from the right side at *. Of the eight specimens shown only one is facing the light, one is vertical, the others facing the dark side of tank.

The two suspended specimens continue as before, one absolutely as at first, the other with a definite crescentic curvature, but forty-five degrees away from light. Fig. 4 is from a photograph taken by Dr. S. R. Williams and gives a good impression of the orientation of such specimens as came within the view. It is taken from the end in order to show the relation of the tubes to light, which came directly from the right and into that side of the aquarium. Of the twenty specimens only eight are shown, and of these only one faces the light, one is almost vertical, the other six incline very definitely toward the dark side of the aquarium.

As will be seen, nothing especially new has developed beyond what has been found in connection with the earlier series. However, since here the conditions of light, temperature, etc., have been so ideal the results not only confirm those already given, but render them more certain and conclusive. It seems quite improbable that three series of experiments directed to a single end should have given uniformly erroneous results; moreover, it is equally improbable that any error of method should have vitiated all three series, varied as these are shown to be, and inspected as they were by several of my colleagues almost from the beginning. Nor is it possible that the matter of season could have been a modifying factor, for it coincided almost exactly with that of Loeb's experiments. That light has been shown to be a wholly negligible factor in relation to the behavior in question has not at any time been claimed. That it has been shown to have only a minor influence I believe the facts conspire to render very certain.

But we are not yet done with the problem. In his original account Loeb cited the behavior of *Spirographis* in the public aquarium as tending to confirm his experimental results "for the most part"! I have studied the problem in this aquarium with especial care during the entire course of my own experiments and have found the behavior to confirm *my* experiments, as the results will show. Let it be expressly understood that in these large exhibition aquaria the best efforts of many years have been directed to render them as nearly natural as it is possible to have such limited portions of the sea; and the fact that some of their occupants have lived and thrived here for more than twenty-five years bears strong evidence to the measure of success in the effort to render them *natural*. In these aquaria *Spirographis* seems to find a fairly congenial environment, and thrives continuously in health for many months. For the sake of exhibition advantages the specimens have been planted, or disposed in such ways as afford the display of the gorgeous, flower-like gills to the best advantage. Hence some are located on the floor of the aquaria, others on the back and ends where rocky ledges afford suitable bases for their support. It ought also to be said that in order to render these aquaria the best possible exhibition cages the illumination is chiefly, and in some case wholly, from above; while the room itself is purposely kept dark, except for the light which diffuses outward from the aquaria. It becomes important that in reference to the problem before us this fact of the source and direction of light be borne in mind. On the assumption of the compelling potency of light it will be clear that in the case under examination there should be a fairly uniformly vertical aspect of the various specimens, whatever may have been their original position. The following are the facts: From several attempts it was determined with approximate accuracy that at this time there were about 150 specimens of *Spirographis* in the aquarium. These were disposed, as mentioned above, on the bottom, ends and back of the tank. Of the entire number about 90 were in more or less vertical attitude or with upward inclination, while 60 were otherwise inclined, that is, they were horizontal or inclining downward. The general facts are fairly well shown in Fig. 3, which is a photograph of the aquarium made by Dr. Sobotta, by whose kind permission I am able to use it in this connection. Of the 60 specimens of this adverse aspect slightly more than half were horizontally disposed, while the others, some 23 specimens, exhibited decidedly downward inclination. The picture will afford excellent illustration, though not taken at the time my observations were made.

Let us now attempt to analyze these facts and their bearings upon our problem. It may be stated at the outstart that gravity has little or no place in the behavior. Loeb has so concluded from his experiments, and my own go to confirm his verdict.

Both in experiments and in nature there seems to be no evidence of its operation. Specimens attach themselves to the bottoms of boats, to overhanging rocks, etc., and seem quite indifferent to its influence. We may therefore proceed to consider the main question at issue, namely, that of light.

Of the 90 specimens having a sub-vertical attitude about 60 were on the bottom of the aquarium, which leaves 30 of this class among those located on the back and end walls. In other words, twice as many of the vertical specimens were located on the bottom as on the sides. But let it be remembered that of the total 150 specimens in the aquarium about 94 were planted on the bottom while only 56 were located on the walls. Further, it is to be noted that those located on the bottom must assume a sufficient degree of elevation to afford a free expansion of the gills; to those on the walls this is not essential. On the other hand, of the 60 specimens which had assumed a horizontal, or downward attitude about 25 were among the bottom specimens, while the other 35 were among those attached to the walls. Expressed in percentages we have the following: Of the whole number about 60 per cent. showed a more or less vertical aspect, while 40 per cent. showed otherwise, *i. e.*, a downward inclination. Of those planted on the bottom about 70 per cent. showed a vertical tendency, and about 30 per cent. were inclined downward. Of those on the walls about 65 per cent. inclined downward, while 35 per cent. inclined toward the vertical.

Now, how shall one interpret these varying aspects? According to theory, "If the rays of light fall vertically from above into the aquarium, *Spirographis* directs its tube vertically upward, exactly as a stem grows vertically up into the air." In the case before us the *light comes vertically from above*, yet a large per cent. of the specimens fail to direct the tubes vertically upward. Of wall specimens 65 per cent. incline downward, or are horizontal in relation to light. Of those on the bottom the per cent. curving downward is much smaller, but still too great to be explained as merely incidental, or by the naive suggestion "Here, however, where free-swimming forms easily disturb the orientation of *Spirographis*, it is not so perfect as when all possible disturbing causes are avoided, as in an aquarium used only for such experiment." Unfortunately for such explanation "free-swimming forms" are rarely present in this aquarium, the only specimens during my observations being the slow and delicate moving little sea horse, *Hippocampus*, whose presence among the relatively colossal *Spirographis* could hardly be of more influence as a disturbing factor than a few sparrows in an oak forest! In fact specimens of *Hippocampus* had been kept for weeks in one of the aquaria in which my special experiments were being made and would frequently attach themselves by their delicate prehensile tails to the tubes of *Spirographis* but without the least evidence of disturbance of any sort. One often finds the tubes of these annelids more or less loaded with tunicates, sponges, hydroids, etc., but there was never any appreciable sign of disturbance therefrom so far as their orientation was concerned.

I think it must be rather obvious that the behavior exhibited by these creatures under the sub-natural conditions of these magnificent aquaria conforms in all essentials with that found in the experimental tanks, and under both these tests there seems to be a fair equivalent of that to be observed in their native habitat.

Concluding Remarks.

The foregoing account, especially when taken as a part of the more extended observations already repeatedly cited ('06, '09), must make it more or less evident that so far from affording any support to the sweeping assumption of the identity of animal and plant heliotropism, based on the behavior of these organisms, strongly *suggests*, if indeed one might not say *warrants*, the very opposite. One might even go a step farther and say that it seems extremely doubtful whether the behavior of *Hydroides*, *Pomatoceras*, *Spirographis*, or any of the tubicolous annelids may be interpreted as an expression of tropisms at all. Without seeking in any way to discredit the possible rôle of light in relation to certain aspects of behavior, it may yet be fairly doubted whether it sustains any such determining influence as has been claimed by the exponents of the tropism hypothesis. Indeed the facts which have been passed in review show beyond reasonable doubt that in relation to these organisms it can have but a subordinate and incidental place. It seems perfectly certain that there is not that degree of constancy, or character of reaction, in orientation which would warrant a tropic interpretation of any sort.

But on the other hand let it not be inferred that behavior is chaotic or beyond scientific explanation. As I have elsewhere pointed out, reactions and adjustments in relation to food-getting, respiration, etc., are among the most fundamental of all phases of behavior. These creatures must live, hence must have food; but they are sessile, and therefore must utilize such as may come within reach. Furthermore, they must respire, and hence must have room within which to expand the gills. All this implies that such colonial species must of necessity frequently resort to movements of readjustment directed to the above imperative ends. In most of these creatures it so happens that one and the same organ is involved in this dual function of food-taking and respiration; a fact of some significance in simplifying or complicating, according to condition, certain phases of behavior. To the writer it seems probable to the point of certainty that the aspects of behavior which have been under review are chiefly but varied expressions of these common functions. In other words, they are aspects of adjustment in the complex struggle for existence— varying modes in which each species has worked out its own special problem of life.

In the light of this mode of interpretation the complicated serpentine torsions of the tubes of *Hydroides* and *Pomatoceras* are the most natural expressions of just such "trial movements" as one might expect. Likewise the bending aspects of the flexible tubes of *Potamilla* and *Spirographis* are not mysterious enigmas over which students of behavior need array themselves in warring camps, but rather the simple expressions of those individual adjustments called for in the varying struggle of life, to the interpretation of which Huxley would have found necessary only "trained and organized common sense"!

I am quite aware that to speak of *individuality*, or *autonomy*, or *spontaneity* as factors involved in problems of animal behavior may to some exponents of mechanism seem "*no explanation*," and of significance only to the psychologist. But as I have earlier pointed out, *they are facts*, and they bulk large in the sum total of animal economy and behavior. To recognize them *as facts* is not to imply thereby their *explanation;* but it *does* imply that they are no less entitled to recognition and explanation than any other classes of facts with which we have to deal. Facts are sometimes characterized as "stubborn things," they have ways of their own; they are tenacious of life; and sooner or later will compel respectful attention and explanation. As is well known, in his matchless account of the behavior of earthworms Darwin did not hesitate to employ a terminology which frankly assumed the presence in these creatures of nervous and psychic factors. While it may not be easy to prove that annelids have a high degree of intelligence, on the other hand he who essays to prove that intelligence has no part whatever in their behavior will hardly have an easier problem.

At no time has the writer questioned the important relations of physico-chemical factors to the phenomena of life and behavior. Further, he has not questioned the possibility of the correlation of these phenomena under *physical laws*, much as we recognize that phenomena of electricity and magnetism and gravitation are conserved under other natural laws. But this by no means implies that these latter species of energy have not their own *special laws*, some of which are already known while others have thus far defied definition and correlation. So, in the matter under review, what he *has* questioned is the very different postilate, that *known properties* of chemistry of physics in any of their *known interactions* afford adequate definition and explanation of *all the facts;* or that *known physical laws*, as applied by the sponsors of mechanism, are convincingly sufficient. It is against the arrogant assumption that a fact of behavior, or an expression of emotion or affection, is never explained till cast into some physical or mathematical formula, that protest has been iterated. In directing attention to the possible interaction of well-known psychic factors in behavior there is merely the plea that similar recognition be given to them as to the former and, as suggested above, they be included in the category of behavior calling for explanation. However independent or unrelated may appear certain of their expressions it is not assumed that in any scientific sense they are mutually exclusive, nor that the one class of phenomena are any less related to causal antecedents than the other. But it *is* maintained that while in some cases these antecedents may be known, and lend themselves to direction and control, on others they are as yet absolutely unknown and more or less beyond prediction or control. And furthermore, it is believed upon experimental evidence that certain aspects of behavior may be more or less variable under any given set of antecedents or conditions; in other words, *given stimuli do not always evoke the same response;* in fact, *much* of *behavior* is *indeterminate* in terms of *existing knowledge*. But so far from implying a reactionary attitude toward the value and importance of continued experimentation, the writer would hold the very opposite. It is well that one pause now and then and take stock in science as well as in business. That problems of behavior are complex beyond earlier anticipation goes without saying. The same must be admitted of every problem of biology. Only the biological pessimist will find occasion to contemplate intellectual suicide because he finds the dogmas of his science in process of revision!

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THE DEVELOPMENT OF THE GONAD AND GONODUCTS IN TWO SPECIES OF CHITONS.

ROSE M. HIGLEY AND HAROLD HEATH.

The later development of the chitons has never been fully investigated, and the fragmentary observations that have been made relate almost exclusively to immature forms in very advanced stages. Accordingly we are at present almost wholly ignorant of the development of the principal systems of organs and their homologies. Many of the more important questions relating to these animals center in the formation of the cœlom, and it was with the hope of throwing some light on this subject that the present work was undertaken.

The two species that form the basis of this investigation, *Trachydermon raymondi* and *Nuttallina thomasi*, are fairly abundant forms in certain localities along the coast of California, and owing to their small size are readily sectioned. The free-swimming young[1] were placed in aquaria together with fragments of shells of *Mytilus californica* on which they finally settled after partially completing their metamorphosis. They were then transferred to small and protected tide pools where they developed normally and in several instances were allowed to reach the sexually mature condition. Precautions were taken to keep the young of each species in separate pools and it was found that they travel essentially the same developmental path for a long period. Distinguishing characteristics accordingly appear late, in fact considerably beyond the formation of the gonad and its ducts. It is to be understood therefore that while the figures are of *T. raymondi* they serve equally well for *N. thomasi*.

At a very early stage the heart and pericardial cavity are developed from cells, giving evidence of being derived exclusively from the secondary mesoblast (progeny of $4D$), which forms an irregular layer on the postero-dorsal side of the larva. A relatively long period of time then ensues, during which the other systems of the body develop to practically the same condition as in the adult, before the gonad makes its appearance. When the primitive sex cells become recognizable they usually form two groups attached to the anterior external surface of the pericardium from which they appear to be proliferated. Very soon, in rare instances at the time of their formation, these become so closely appressed as to appear single though sections show them to be distinct for a considerable time, frequently after the gonoducts have formed. Shortly after their appearance a cavity forms within each group, and, with the growth of the gonad, soon becomes more or less triangular. In later stages, generally about the point of development represented in the figure, these cavities gradually fuse, commencing at the posterior end of the gonad and progressing anteriorly. In some individuals a slight groove may persist on the ventral surface between the halves of the gonad for a considerable time, and in a few cases a distinct cleft at the anterior end of the gland persists until the animal is half grown.

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[1] For breeding habits of these species see *Zool. Anz.*, Bd. XXIX., No. 12.

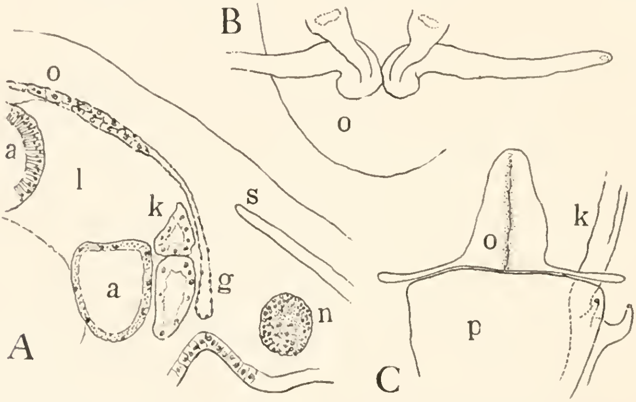

FIG. 1. Gonad and ducts of *Trachydermon raymondi*. *A*, section through animal about 1 mm. long. *a*, digestive tract; *g*, gonoduct connecting with gonad; *k*, kidney; *l* liver; *n*, lateral nerve cord; *s*, shell. *B*, gonad (*o*) and ducts in mature animal, dorsal view. *C*, reconstruction of same stage as Fig. 1. Gonad with ducts ending blindly; kidney showing reno-pericardial and external openings.

The aorta holds the normal position on the dorsal surface of the gonad, and there are slight evidences that a portion of the blood it carries makes its way between the halves of the organ as in the solenogastres. At all events there are no signs of distinct branches penetrating the gland as in the later stages.

About the time of the fusion of the gonad cavities (when the length of the body is approximately 1 mm.), in a stage slightly earlier than the one represented in Fig. 3, each gonoduct arises as a slender evagination of the postero-lateral walls of each half of the reproductive gland. These grow rapidly, and in contact with the pericardial wall proceed laterally and ventrally until they come in contact with the ectoderm of the mantle groove. In the formation of the outer opening the ectoderm cells appear merely to separate; if an ectodermic diverticulum is formed it is evidently very short and transitory.

In later stages the proximal ends of the gonoducts shift forward slightly, and are attached to the dorsal side (Fig. 2) of the gonad close to the mid line. During this process their walls thicken, and at the height of the breeding season there are signs of secretory activity on the part of the component cells especially in the neighborhood of the reproductive organ. The eggs of both of these species are held in the mantle cavity, and are loosely bound together possibly by this secretion of the oviduct.

The only other observations bearing on the development of the gonoducts are those of Plate[1] who has made the claim that in the young of *Acanthopleura echinata*, 15 mm. in length, the gonad is completely separated from the gonoducts that, as slender diverticula, are connected with the mantle cavity and are accordingly ectodermic. Granted that this is the true state of affairs in *A. echinata* it is unprofitable for the present to attempt to correlate the two types of development when only three species of chitons have been examined on this point. However, it is interesting to note that in several species of California chitons[2] three millimeters or less in length the gonad and its ducts are attached and open to the exterior. In some species, such as *Ischnochiton magdalenensis*, the ducts are highly glandular and it is possible, though it appears to us improbable, that this glandular section is of ectodermic origin.

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[1] *Zool. Jahrb.*, Suppl. 4 (Fauna Chilensis, Vol. 1).

[2] Heath, *Zool. Jahrb.*, Bd. 21, p. 729.

ASTEROPHILA, A NEW GENUS OF PARASITIC GASTROPODS.[1]

JOSEPHINE RANDALL AND HAROLD HEATH.

During the dredging operations of the U. S. F. C. Str. *Albatross* in the vicinty of Japan (summer of 1906) four specimens of a starfish, *Pedicellaster* sp., were taken that had been parasitized by a new genus of gastropods. All were dredged in the sea of Japan off the coast of Corea at depths ranging from 150 fms. (sta. 4,867) to 163 fms. (sta. 4,861). In one host three parasites occurred, while only one was present in each of the other three, but in any event they occupied the cœlom in the arm, and were attached by connective tissue strands to the body wall in the vicinity of the ambulacral ridge. As noted more particularly hereafter, this species is not put in communication with the exterior, the mouth and reproductive openings communicating with the body cavity of the host. During the time that the brood pouch is crowded with embryos the pseudollapium becomes accordingly considerably distended and tense, resulting in the inflation and consequent thinning of the body wall of the host along the dorsal side of the arm (Pl. II., Fig. 2). Under such circumstances it is possible that the body wall of the starfish finally ruptures, causing a diminution of the pressure on the pseudopallium which therefore discharges the embryos into the surrounding medium. After this process the break in the body wall is probably repaired, as there are evidences that one of the larger individuals has recently discharged its brood though there are no signs of a rent in the starfish arm.

In every case the body resembles in form a kidney or thick-set bean, and varies in size from two to twenty millimeters, this last extreme being due to some extent to the large number of embryos and the fluid in which they float. The ovary and the embryos themselves are light yellow in color due to the presence of yolk, the liver is of a light brownish shade while the other organs are unpigmented and more or less translucent, especially in the case of the pseudopallium that in life is so thin and transparent that the form and movements of the larvae may be readily observed. As may be seen in Pl. I., Fig. 1, there are two openings into the body, one the mouth, corresponding in position to the hilum of a bean while the reproductive opening is placed laterally upwards of thirty degrees.

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[1] Published by permission of Hon. G. M. Bowers, Commissioner of Fisheries.

From various features of its organization it is readily possible to orient this animal and discover the axes of the body. As in several other parasitic gastropods the body is surrounded by a pseudopallium that appears to be a development of the snout or adjacent regions of the body. Growing upward it has enveloped the body completely save at one point, the reproductive and excretory pore. Considering the body proper, the foot is seen to exist in the form of a small though broad wedge-shaped fold (Pl. I., Fig. 1, *f*) covered with epithelium of greater thickness than that surrounding the body generally. In sections (Pl. II., Fig. 4, *f*) it is a fairly conspicuous object owing to its affinity for stains. Again, well-defined pedal ganglia and otocysts, located in close proximity to the foot and cerebral ganglia on the opposite side of the digestive tract, demonstrate the fact that the antero-posterior axis is the shorter of the roughly ellipsoid body, and that the transverse axis is accordingly the longer.

In this species the degenerative processes have advanced to a stage where the mantle and mantle cavity have largely disappeared, and yet, though rudimentary, they maintain their typical relations. Since the mantle fold is comparatively narrow (Pl. I., Figs. 1, 2, *g*) the cavity is accordingly shallow, as the mantle is closely applied to the visceral mass; nevertheless the epithelial cells bounding the cavity are not only higher than those elsewhere covering the body but they stain more intensely and are ciliated. On the left-hand side of the body the mantle border thickens considerably, and forms a projecting ridge that continues until the pallial cavity itself disappears. In the smallest specimen the mantle and cavity are relatively larger and the mantle fold is much more glandular, the gland cells being large and conspicuous.

As noted in a preceding paragraph, the mouth opening is borne on the summit of a low papilla in the mid line. In entire specimens it is further distinguished from the opening into the pseudopallium by occupying the center of a whitish area, upwards of 3 mm. in diameter in the largest specimens, caused by the compact feltwork of circular and radiating muscles enveloping what probably corresponds to the buccal tube. In the immediate neighborhood of the mouth opening the canal is comparatively slender, 0.28 mm. in diameter in large individuals, and is provided with a lining of simple columnar cells whose distal portions contain small quantities of a faintly staining, vacuolated secretion. Behind this point large numbers of small, irregularly distributed pyriform gland cells appear imbedded in the muscular meshwork surrounding the digestive tract, and their darkly staining ductules may be traced to intercellular openings in the buccal or pharyngeal epithelium, whose extent is increased by two symmetrically placed diverticula with short, stubby branches (Pl. I., Fig. 2) extending a short distance into the surrounding muscle sheath. These paired glands probably correspond to the ventral salivary glands of other molluscs as the buccal ganglia, connected by a commissure, are located in their vicinity.

No trace of a radula exists.

The buccal-pharyngeal tube with its enveloping glands and muscles, is relatively short, probably not over 1 mm. in length, but it spans a well defined head cavity (Pl. II., Fig. 3), which is a portion of the hæmocele as in other molluscs. Curving gently toward the ventral side of the body the tube leaves the sinus, and now devoid of gland cells and with a comparatively thin sheath of longitudinal and circular muscles, it passes back a short distance into the body and unites with the main portion of the digestive tract (*l*), a spacious cavity, lined with glandular epithelium, occupying most of the visceral mass not held by the gonad and its duct.

The pericardial cavity (Pl. I., Fig. 2) is situated on the anterior surface of the visceral mass on the right side. The contained heart consists of a single auricle and ventricle, both of large size and highly muscular. The first-named receives the blood from a broad sinus, which on one hand passes from the liver surface and the neighborhood of the accessory reproductive glands in the ventral part of the visceral mass, and by means of another smaller branch takes the blood from the kidney. The aorta is very short and leads directly into what may be termed the head cavity, the large space surrounding the pharynx. From here numerous branches extend into the pseudopallium, liver and between the ovarian follicles. Of these the ones passing through the pseudopallium probably function in the interchange of gases as there are no traces of ctenidia or branchia.

One nephridium (Pl. I., Fig. 2, *n*) is present in the form of a greatly compressed sac covering the anterior surface of the visceral mass on the right-hand side. Its inner walls are often provided with lamellæ or folds, of varying size, projecting into the central lumen. The cells throughout are highly vacuolated and contain varying quantities of some granular secretion that in some locations present the form of concrements. We have been unable to definitely locate any clearly defined reno-pericardial opening. As shown in Pl. II., Fig. 6, *n*, the kidney invests the dorsal pericardial wall but there are, so far as we have seen, no modified cells indicating a nephrostome. The external pore (Pl. I., Fig. 2, *e'*) is situated on the anterior face of the visceral mass immediately below the margin of the mantle.

While the ganglia are fairly well defined and distinct the nerve fibers resemble so closely the connective tissue and muscle bundles through which they make their way that it is very difficult to determine their course. The cerebral (Pl. I., Fig. 1, *c*), apparently associated with the pleural, are, in the type specimen, situated in an asymmetrical position, being placed on the right side of the pharynx. From this nerve mass connectives extend, on each side of the pharynx, to the pedal ganglia, large, closely appressed groups of nerve cells placed symmetrically with reference to the mid ventral line. The cerebral ganglia likewise originate buccal connectives that, extending along the pharynx, unite with ganglia imbedded in the salivary glands on the dorsal and ventral side. The buccal ganglia are further united by two commissures that form a collar about the pharynx. In the neighborhood of the opening of the reproductive system into the pseudopallium there is a large ganglion, probably the visceral, that gives off a strong nerve which may be traced a short distance posteriorly, and in the opposite direction a single connective leads from it to the cerebro-pleural ganglia, as indicated in Pl. I., Fig. 1, *v*. At various points throughout the body it is possible to discover nerve bundles, but in every case it has been impossible to determine their origin.

In the three animals examined there is no sign of a testis, though the seminal receptacle of one individual contains a considerable number of spermatozoa. These last named elements possess almost spherical heads measuring approximately 0.004 mm. in diameter. Nuclei of somewhat similar appearance may be detected here and there in the follicles of the reproductive gland, but their close resemblance to those of the connective tissue cells renders the determination uncertain. If self-fertilization does not occur in this species it is difficult to understand the method of sperm transfer especially in those examples where but one parasite occurs in the host which completely envelops it.

The ovary, occupying fully half of the visceral mass, consists of a large number of follicles united directly or indirectly with the duct leading to the exterior. In a mature condition the central portions of each follicle are packed with fully developed ova, while numerous cells in the earlier stages of formation bound the periphery. About the center of the visceral mass the common chamber, communicating with the ovarian follicles, narrows anteriorly and the short resulting tube, after a somewhat twisted course, unites (elliptical stippled outline, Pl. I., Fig. 2) with the definite gonoduct leading to the exterior. This last-named canal consists of three divisions corresponding to the albumen and mucous glands and the seminal receptacle in other species of gastropods. The canal from the ovary unites with the albumen gland which extends posteriorly as a pouch of considerable size. Its walls are relatively thick, and are fashioned into a few prominent folds, consisting of relatively slender cells, whose vacuolated secretion stains lightly with Delafield's hæmatoxylin. Slightly anterior to the oviduct connection, a cone-shaped seminal receptacle (Pl. I., Fig. 2, *r*) is attached to the albumen secreting section. Its epithelial lining is developed into a large number of folds between which there are quantities of spermatozoa, that likewise occupy the main lumen and even extend in small quantities some distance into the albumen gland. Anterior to the seminal receptacle the walls of the canal change abruptly, becoming thicker and the secretion stains so intensely that the cell outlines and nuclei become almost completely obliterated. This state of affairs exists between the seminal receptacle and a point slightly posterior to the external reproductive opening. Anterior to this region the duct presents the form of a roughly conical sac extending to a point opposite the foot. The walls of this pouch are similar to the darkly staining ones just described save that the secretion is more vacuolated and accordingly less deeply stained. The duct leading from this mucous secreting, main canal to the exterior is relatively short, thin-walled and passes into the furrow at the right side of the body formed by the union of the visceral mass with the pseudopallium.

In two specimens whose pseudopallium contained fully 500 embryos the ovary held an equal number of ova in a fully developed condition. Hence it is probable that during adult life the brood pouch is empty for short periods only.

The genus may be defined as follows:

Asterophila new genus. Body globular, 2–20 mm. in diameter, completely enveloped in the pseudopallium. Foot and mantle rudimentary. Buccal-pharyngeal tube, with salivary glands, opens into combined stomach and digestive gland that otherwise do not open to the exterior. No radula. Albumen and mucous glands on reproductive canal highly developed, and seminal receptacle prominent. Parasitic in starfish *Pedicellaster* sp., Sea of Japan. Type of genus *A. japonica*.

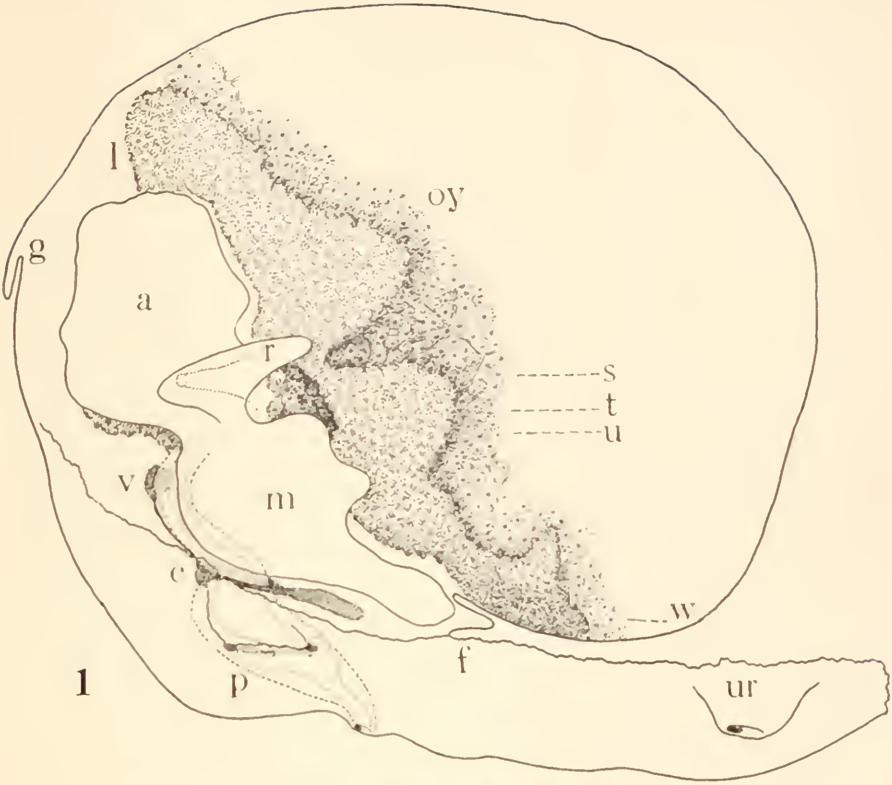
A. japonica new species. With characters of the genus.

EXPLANATION OF FIGURES.

PLATE I.

FIG. 1. Diagrammatic view of *Asterophila japonica*, left side, with the greater portion of the pseudopallium removed. *a*, albumen gland; *c*, cerebral ganglion; *f*, papilla-like foot; *g*, mantle fold; *l*, digestive gland or liver; *m*, mucous gland; *oy*, ovary; *p*, buccal tube and pharynx with salivary glands, buccal ganglia, connectives and commissures; *r*, seminal receptacle under which is dotted outline of duct from ovary; *ur*, urino-genital opening; *v*, visceral ganglion.

FIG. 2. Diagram of anterior surface. *e*, *e′*, openings of reproductive and excretory systems into pseudopallial space; *g*, mantle fold, the depth of the mantle cavity indicated by broken line; *h*, heart; *n*, kidney; *p*, buccal tube; *r*, seminal receptacle.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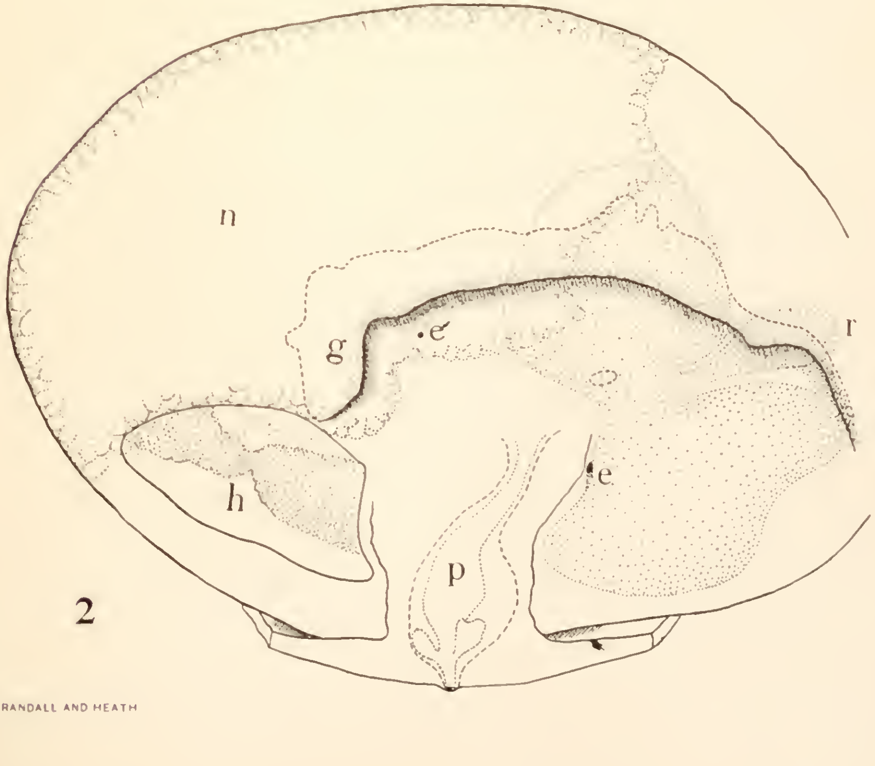

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PLATE II.

FIG. 1. Anterior view of *Asterophila japonica* with pseudopallium partially removed.

FIG. 2. Arm of starfish containing parasite.

FIG. 3. Section through pharyngeal tube, showing salivary glands, buccal ganglia and surrounding head sinus.

FIG. 4. Section through foot and visceral mass; along line *w* of Pl. I., Fig. 1.

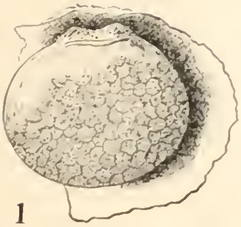
FIG. 5. Section along line *u*, Pl. I., Fig. 1; the junction of the œsophagus and stomach-intestine marked by an arrow.

FIG. 6. Section along line *t*, Pl. I., Fig. 1.

FIG. 7. Same along line *s*, Pl. I., Fig. 1.

FIG. 8. Diagram illustrating growth of pseudopallium in *Asterophila*.

FIG. 9. Same, *Ctenosculum hawaiiense*.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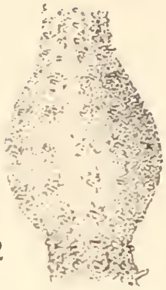
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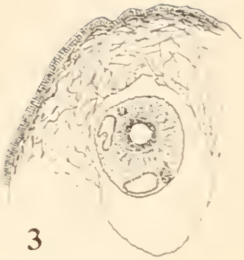
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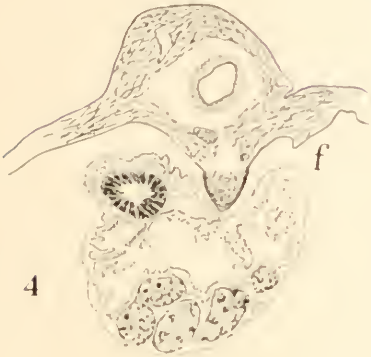

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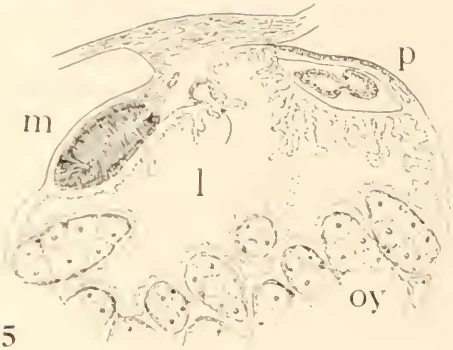

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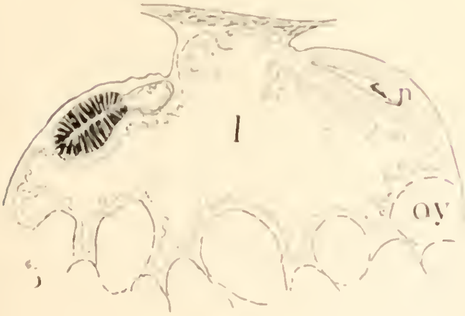

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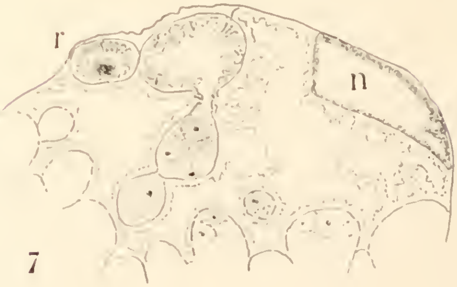

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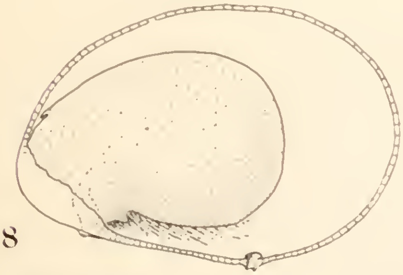
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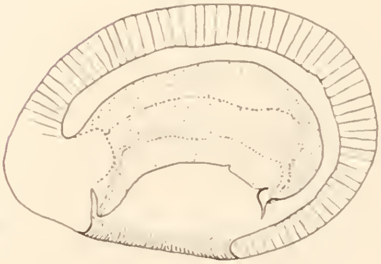
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A CASE OF YOLK FORMATION NOT CONNECTED WITH THE PRODUCTION OF OVA.

OSCAR RIDDLE.

The secretion or production of true yolk in situations other than in ova or in the follicular cells which surround ova is not known so far as I am aware. "Nurse" or "yolk" cells have of course long been known to exist in several groups of animals; these, however, are evidently the equivalents of follicular cells or of ova. It is therefore of some interest to record the finding of true yolk in spaces within the connective tissues which lie externally to the follicular membrane of capsules which had previously liberated ova.

These observations were made on the ovaries of the common fowl during the mid-summer season. During July and August of the present summer the writer had occasion to examine the ovaries of more than one hundred full-grown hens. Among these there were at least six or eight ovaries which showed unmistakably the peculiar accumulation and placement of yolk which is here described.

In order to be sure that one is really dealing with "extra-ovular" and "extra-follicular" formation of yolk, and not merely with a masque of its usual source, it was necessary to determine three things concerning the capsules within which the yolk in question was found: (1) That an egg had been surely developed and liberated from this capsule. (2) That the space in which the yolk was found is quite separate and removed from the space formerly occupied by the ovum, and likewise removed from the follicular cells which surrounded the ovum. (3) That the accumulated substance is true yolk. I believe that the material I have examined has enabled me satisfactorily to determine each of these points.

The evidence that the yolk-containing capsules in question had previously borne and liberated ova rests partially upon the finding of capsules showing all the intermediate stages between the recently broken capsules and the large, flabby, often asymmetrical, yolk-containing ones. Some of these latter capsules might be mistaken for resorbed ova, since they too have a closed stigma; that is, the slit or splitting which occurs in the capsule at the time of ovulation, and which allows the escape of the ovum, later heals together and the cavity of the follicle is once more completely sealed. The chances for such confusion are further increased by the fact that this central chamber may also occasionally re-accumulate yolk.

It is possible nevertheless in favorable material to be quite sure that the stigma has been *broken* and reunited—a thickened, accentuated, and often more or less ragged point of reunion indicating this. Furthermore, a series of follicles in the *same* ovary, showing the most recent ones still broken open, often decides the matter at once with certainty. The capsule from which a sample of yolk for analysis was taken was one of such a series. In this case there were nine yolk-containing capsules in various stages of extra-ovular yolk-production; and in addition, one other—the newest follicle—plainly recognized by its whole appearance as a recently emptied one. This follicle, however, showed the once *broken lips of the stigma now nearly completely grown together*, but with its inner cavity as *clean and free from yolk* as at the moment of ovulation. It is certain that the follicles of this ovary had liberated ova, and that instead of degenerating thereafter these capsules quickly closed the breaches formed in extruding the ova, and began the production of yolk in their external walls.

It is easy to demonstrate that the yolk-filled spaces bulging from the sides of the capsules have no open connection with the central cavity of the capsule; that is to say, these spaces are not connected with the former seat of yolk formation. Several times I have made a slit in the scar or stigma and, finding the interior clean and free from yolk, have tried by squeezing the various bags of yolk lying in the external walls of the capsule to make their yolk flow into the central cavity. In no instance have I succeeded in thus finding any connection whatever between these new yolk-containing cavities and the old cavity formerly occupied by the egg. On the contrary, careful dissections of these capsules show that the two spaces are always separated by a rather thick wall; certainly much thicker than that which separates the new yolk space from the exterior. This latter wall, in fact, is usually very thin. It consists, however, of an extremely thin connective tissue layer in addition to the ovarial epithelium. By careful handling the epithelium can be stripped off and the thin layer enclosing the yolk space left intact.

The very external position of the yolk spaces—of which there may be several in a single capsule—makes it evident that none of the cells of the old follicular membrane are engaged in the production of yolk in this new and unusual site. The production of this yolk is necessarily accomplished by the cells which form the external theca—a tissue from the former ovarian stroma, which in the late growth stages of the capsule of large eggs becomes a very thick, firm, essentially connective tissue layer enclosing possibly some scattered derivatives of the germinal epithelium—whose cells normally take no part in yolk formation.

Just what it is that transforms these non-yolk-producing cells into cells actively engaged in yolk production, it would be most interesting to know. While confessing very complete ignorance as to this cause, it seems worth while to note that *the cells which here take up a new function do so at the time when the "normal" thing for them to do is to degenerate and be absorbed.*

In this connection it should be stated that the true follicular cells—those which have previously been engaged in passing on the constituents of yolk to the egg—are apparently the least liable of any of the capsular cells to take part in any later yolk production. Only occasionally in a group of capsules, each of which may be producing yolk at one or more points externally, will one find that the follicular cells have continued—or rather have recommenced—to produce yolk. What I have observed would indicate that these follicle cells never in any case become active until after yolk production has been initiated in the more external layers; but of this latter point I am not certain.

That the yellowish fluid enclosed in these yolk spaces is true yolk is indicated by its microscopic appearance. The question is positively and affirmatively answered by the chemical analysis of a sample. 1.605 grams of such yolk were collected from a single one of the new yolk spaces; this was not all, but nearly all of the contents of the cavity. In order to show how closely its chemical composition agrees with that of other forms of true yolk, I have added to the table the numbers resulting from the analysis of four such samples of yolk. Reference to the table readily shows the essential similarity of all these substances; and likewise a point or two of notable difference.

Analysis of:	In Per Cent. of Solids.					H_2O.
	Lecithin.	Protein.	Neutral Fat.	Total Ash.	Organic Extractives.	
Extra-follicular yolk	19.05	26.21	45.39	6.61	2.65	74.22
Central "yolk body" from incubated hen's egg	19.68	28.87	46.05	3.40	2.00	37.13
Egg yolk, Jungle fowl	19.90	30.47	46.74	1.30	1.59	48.70
Contents of yolk-sac; 21 days incubation	17.62	33.24	47.36	1.16	1.39	56.52
Resorbed ovum	15.30	35.18	42.25	1.71	2.08	63.20

It is true that I have selected for this comparison analyses which most closely agree with the analysis of the "extra-follicular" yolk. The high water content of the latter is of no consequence; an analysis of "white" yolk from the hen having yielded more than 80.0 per cent. of this constituent.

The high ash content, and very low protein content, do indicate however a species of yolk not in all respects like that produced by the follicular cell and the ovum. In these two respects this yolk stands as a rather bold extreme in a long series of analyses of normal yolk. It can be said therefore that though this substance is certainly "yolk," its peculiar origin stamps its chemical composition with a specificity of its own.

The foregoing recital of the facts is perhaps hardly sufficient to uncover at once to every reader one of the points of interest in these findings; at any rate it is a point of interest to the writer. I refer to the fact that in all of the hitherto known cases of yolk formation the whole process of yolk building and storage appears so glaringly and profoundly teleological. The ovum prepares and stores food for an embryo that is yet to form; a follicular cell passes on this rich material only to an ovum which in turn accumulates for a promised organism that will arise and require the store; ovum and ten thousand follicular cells unite to prepare and to hoard a pabulum for an organism whose father exists as yet only in prophecy and in fortune; a "nurse" cell arises in a distant part, migrates with its supplies and unerringly delivers all to the egg—whose prospective accomplishment only can use or require them; or, again, as in some hydroids, several adjacent ova laboriously produce a golden store which together with their own existence they place sacrificially upon the altar of posterity—giving all to a more opulent neighbor, who through the combined accumulations of many gatherers can the more adequately and assuredly provide for the beginning of an individual that is to be.

Nor is such apparent teleology absent from the very chemical composition of the material that is stored. The developing organism requires above all else a store and source of energy, and one notes that yolk—the material actually stored—is richer in lecithin and fat than is any other product of the body; and further that these constituents are the ones which carry far more energy per unit of weight or volume than do any others.

When, however, one turns to the sort of yolk formation described in this paper,—yolk formation which begins in subdued and atretic follicles, among cells largely "somatized" and doomed to certain degeneration; when one considers the utter blindness involved in these ill-conditioned cells plunging into a most active production of excessively rich foods, only to cast them into the formless spaces of these spent capsules, one can realize that the process of yolk building actually can be as grotesquely absurd and inappropriate as it has elsewhere seemed replete with insistent teleology.

Laboratory of Experimental Therapeutics,
The University of Chicago,
September, 1911.

Vol. XXII. *February, 1912.* *No. 3*

BIOLOGICAL BULLETIN

THE OSMOTIC AND SURFACE TENSION PHENOMENA OF LIVING ELEMENTS AND THEIR PHYSIOLOGICAL SIGNIFICANCE.[1]

J. F. McCLENDON.

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Preface.

This paper formed the basis for two lectures given before the class in physiology at Woods Hole, July 7 and 8, 1911, although owing to limited time, some parts were omitted. Since then there has appeared a second edition of Höber's "Physikalische Chemie der Zelle und Gewebe," which reviews much of the literature considered in this paper. However, owing to an entirely different mode of presentation, it is hoped that the present treatment of the subject might be helpful to many general readers, some of whom would not read Höber's book.

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[1] From the Embryological Laboratory of Cornell University Medical College, New York City.

I am indebted to several persons for suggestions, especially to Dr. Ralph Lillie[1] and Professor B. M. Duggar.

I. INTRODUCTION.

The object of this paper is to bring the "vital" phenomena, as far as possible, within the scope of physics and chemistry, and not to elucidate physical and chemical processes. It should therefore be borne in mind that the osmotic phenomena of "dead" systems are not all satisfactorily explained.

The Vant Hoff-Arrhenius theory of osmosis concerns itself with the number of particles, molecules and ions, in solution, and is applicable to dilute solutions, in which the total volume of the dissolved particles is negligible. However, in more concentrated solutions, the volume of the dissolved particles is of the same importance as the volume of the molecules in gases, as expressed in Van der Waal's equation. Also the dissolved particles bind molecules of the solvent and so reduce the volume of the free solvent.

That the molecules and ions of a dissolved substance bind some molecules of the solvent, follows from the work of Jones and his collaborators.[2] Compare also the work of Pickering.[3] Jones concludes that the larger the number of molecules of water of crystallization, the greater the hydrating power of a substance in aqueous solution. The number of molecules of water bound by one molecule of the solute usually increases with dilution up to a certain point (the boundary between concentrated and dilute solutions, beyond which there is no heat of dilution). The bond between ions and the solvent is also indicated by the phenomenon known as "electrical transference." If an electrolyte and a non-electrolyte be dissolved in water and an electric current passed through the solution, water will be carried along with the ions to the electrodes.

With these corrections, the Vant Hoff-Arrhenius theory accounts for osmotic pressure, but does not show why many substances exert no osmotic pressure, in other words, why no membranes have been found that are impermeable to them. Overton supposed that the substance, in order to diffuse, must dissolve in the membrane. Kahlenberg and others consider a solution as a chemical combination between solute and solvent, and osmosis as a series of chemical reactions between the membrane and the two solutions, continuing until equilibrium is established. The essential points in the theory are: that the membrane is not a molecule sieve, but a substance with specific properties, and the chemical characters of the membrane and of the dissolved substances affect osmosis.

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[1] Cf. this journal, 1909, XVII., 188.

[2] "Hydrates in Aqueous Solution," Pub. No. 8, Carnegie Ins. Wash., 1907.

[3] Whetam, "The Theory of Solution," 1902, Cambridge, p. 170.

Willard Gibbs found that the more a solute lowers the surface tension of a solution, the more it tends to pass out of the solution, *i. e.*, by osmosis, or if this is prevented, to collect at the surface of the solution. This law has been extensively investigated and confirmed by I. Traube. For instance, in general, lipoid-soluble substances lower the surface tension of water and tend to diffuse out of it, whereas electrolytes slightly raise the surface tension of water and attract water from the adjacent phase. Osmosis may occur in opposite directions simultaneously. Gibbs and Traube state that the greatest osmotic flow is from the solution of lower surface tension to that of the higher, but this is not generally accepted. Osmosis consists of two distinct processes, from one solution to the membrane, and from the membrane to the second solution.

In case the membrane consists of two or more chemically different membranes placed one on another, osmosis consists of a series of steps; and Hamburger[1] made double membranes through which certain substances diffuse more rapidly in one direction than in the other.

Traube calls the bond between solute and solvent the "attraction pressure." In general, attraction pressure of ions increases with valence. The less the attraction pressure of the solute, the more it lowers the surface tension and tends to pass out of the solution. The presence of one solute lowers the attraction pressure of another in the same solution, and the greater the attraction pressure of a solute the more it lowers that of another. We might express this idea by saying that one substance takes part of the solvent away from the second and increases the concentration of the second substance. This may explain the effect of a harmless substance in increasing the toxicity of a poison. Schnerlen[1] observed that a solution of phenol below the threshold of toxicity for certain bacteria is rendered toxic by adding NaCl. Stockard showed that the toxicity of pure solutions of salts on fish eggs is increased by the addition of sugar, although the total osmotic pressure of the mixture is less than that of the normal medium.[2]

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[1] *Biochem. Zeit.*, 1908, XI., 443.

Just as Traube's precipitation membranes are absolutely impermeable to certain substances, so do living cells show this selective permeability. For instance, the vacuole fluid or cell sap of certain plant cells contains colored substances which do not diffuse into the protoplasm surrounding the vacuoles. If a cell be placed in a solution of the pigment, the protoplasm remains colorless. If the protoplasm be squeezed out of the cell into a solution of the pigment, it does not invariably become stained. However, if the cell is injured in certain ways, or dies from any cause, the pigment diffuses out of the vacuoles into the protoplasm and thence into the surrounding medium. We might conclude that the protoplasm in general is impermeable to the color, but at death it becomes permeable. On the other hand, Pfeffer[3] gives evidence for the existence of a mechanical membrane on the surface of the cell and lining the vacuoles. De Vries[4] placed cells into 10 per cent. KNO_3 solution colored with eosin. The plasma membrane and granular plasm died and stained long before any dye entered the vacuoles. However, the granular plasm may have absorbed all the dye, thus preventing its entrance for some time, without the necessity of any resistance of the vacuole membrane. Since protoplasm may be squeezed out in the form of droplets and still appears to be surrounded by membranes, Pfeffer concluded that the membrane was formed by the contact of the protoplasm with the medium or with cell sap. He supposed these membranes to be the semipermeable parts of the cell, and that they became altered at death. Pfeffer called this membrane on the cell surface the "plasma membrane."

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[1] *Arch. exp. Path.*, 1896, XXXVII., 84.

[2] However the NaCl in Schnerlen's and sugar in Stockard's experiment may have increased the permeability to the toxic substances, as discussed in later chapters.

[3] "Pflanzenphysiologie."

[4] *Jahrb. wiss. Bot.*, 1885, XVI., 465.

Whereas the nuclear membrane and certain vacuole membranes are semipermeable, these are lacking in erythrocytes, which are therefore good objects for testing the question whether the protoplasm in general, or merely its surface, is semipermeable. Höber[1] by two very ingenious but complicated methods, one based on dielectric capacity, determined the electric conductivity of the interior of the erythrocyte without rupture of the plasma membrane. Since the conductivity of the interior (about that of a .2 per cent. NaCl solution) was found to be many times greater than that of the erythrocyte as a whole, the membrane must be relatively impermeable to ions. There is much other, but less direct, evidence that the semipermeability resides in the plasma membrane, namely: the rapidity of change in permeability of certain cells, the sudden increase in permeability of a cell after swelling to a certain size (due presumably to rupture of the plasma membrane), the ease with which mild mechanical treatment increases the permeability, and the localization of electric polarization at the cell surface.

Quincke[2] supposed these membranes to be of a fatty nature. This idea was carried further by Overton, who considered the plasma membrane to be composed, not of neutral fats, but of substances of the class which are called "lipoids," which included non-saponifying ether soluble extracts of organs, *i. e.*, cholesterin, lecithin, cuorin, and cerebrin. He found[3] that all basic dyes were easily absorbed by living cells, but not most of the sulphonic acid dyes. This corresponded to their solubility in melted cholesterin, or solutions of lecithin and cholesterin, or particles of lecithin, protagon or cerebrin. His argument is somewhat weakened, however, by the fact that cholesterin decomposes on melting, and that if lecithin is allowed to absorb water its solvent power changes.

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[1] *Arch. f. d. ges. Physiol.*, 1910, CXXXIII., 237, and Eighth Internat. Physiol. Congress, Vienna, 1910.

[2] *Sitzber. d. Kon. Preuss. Akad. d. Wissensch. zu Berlin*, 1888, Bd. XXXIV.

[3] *Jahrb. wiss. Bot.*, 1900, XXXIV., 669.

Many of Overton's critics do not distinguish between lipoids proper and a host of ether-soluble substances which are also called lipoids, and of the data which they present we will consider only that on lipoids proper. Ruhland[1] found that certain dyes stain plant cells but are not soluble in solutions of cholesterin (and vice versa). Robertson[2] observed that methyl green freed from methyl violet was insoluble in a nearly saturated solution of lecithin in benzol, whereas it stained living cells. Höber[3] obtained Ruhland's results, when using certain animal cells, but found that certain nephric tubule cells absorb all dyes that are not suspension colloids.

Faure-Fremiet, Mayer and Schaeffer[4] state that pure cholesterin does not stain with any dyes (contrary to Overton), malachite green (considered lipoid-insoluble by Ruhland and Höber) stains lecithin, and Bismarck brown (considered lipoid-insoluble by Ruhland) stains lecithin, cholesterin-oleate and cerebrin. A mere trace of free fatty acid greatly affects the behavior of lipoids toward stains.

Mathews[5] considers the absorption of dyes by cells as a chemical process. Since basic dyes combine with albumin in alkaline solution, lipoids in the membrane are not necessary for the absorption of such dyes.

Traube objected to Overton's hypothesis on the ground that Overton's plasmolytic series is the same as found by Brown, who used the membrane of the barley grain,[6] and the same as the series of the attraction pressures of the substances in water. But Traube admits in his later papers that the chemical character of the membrane affects osmosis.

We may conclude that, although the plasma membrane of some cells may be lipoid in character, this has not been proven, but, in general, it is more permeable the more the diffusing substance lowers the surface tension of water.

[1] *Jahrb. wiss. Bot.*, 1908, XLVI., 1, and *Ber. Deutsch. bot. Gesellsch.*, 1909, XXVI., 772.

[2] *Jour. Bio. Chem.*, 1908, IV., 1.

[3] *Biochem. Zeit.*, 1909, XX., 55.

[4] *Arch. d'Anat. Mic.*, 1910, XII., 19.

[5] *Jour. Pharmacol. and Exp. Ther.*, 1910, II., 201.

[6] But this is not true of all seed coats. Atkins, *Sci. Proc. Roy. Dublin Soc.*, XII., n. s., No. 4, p. 35, observed that the membranes of the bean seed are freely permeable, semipermeable plasma membranes arising only after germination.

Nathanson[1] supposed the plasma membrane to be a mosaic of lipoids and "protoplasm," but it is evident that if the lipoid portion is not continuous, it can not make the cell impermeable to any substance.

Czapek[2] states that lipoid solvents cause cytolysis when the surface tension of the solution is reduced to .68, and concludes from this that the plasma membrane contains glycerine tri-oleate since its emulsion reduces the surface tension of water to this figure.

The diffusion of water-soluble substances through swollen-plates, "gels" or "sols" of gelatine, varies inversely with the viscosity (Arrhenius). The great hysteresis of gelatine gels is taken advantage of to show that diffusion depends on the viscosity and not on the per cent. of gelatine, at the same temperature.[3]

The absorption of water by a gelatine plate increases its permeability, and the temperature and therefore the presence of substances which affect this swelling of gelatine affect its permeability. Impregnation of colloidal membranes with bile salts, alcohol, ether, acetone or sugar changes (usually increases) their permeability. The effects of substances on the rate of diffusion through gelatine plates, and on their swelling (viscosity) and melting point are not always quite parallel.[4]

In case the substance added to the membrane is removable, the change in permeability becomes reversible, which is true in regard to many of the substances mentioned above. Changes in non-living membranes are usually only partially reversible or are irreversible. Denaturalization of a colloid membrane by heat, heavy metals, or other coagulative agents which induce chemical changes in the membrane, or the addition of substances which cannot be removed, produce irreversible changes in permeability.

That the permeability of the membranes in living tissue is increased at death is proven by a host of observations. The electric conductivity increases enormously at death. Contained substances diffuse out, substances in the medium (fixing fluids, stains, etc.) diffuse in. There is a more general mixing of tissue substances. Enzymes come in contact with proteids and autolysis results.

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[1] *Jahrb. wiss. Bot.*, 1903, XXXVIII., 284; 1904, XXXIV., 601, and XL., 403.

[2] *Ber. deutsch. bot. Gesell.*, 1910, 28, 480.

[3] Zangger, Asher & Spiro's *Ergeb. der Physiol.*, 1908, VII., 99.

[4] Zangger, *loc. cit.*

Certain substances are known to increase the permeability of membranes in tissues of the body. Thus ether, chloroform, etc., increase the penetration of fixing fluids, and the exit of contained substances, and the mixing of tissue substances. In this way they increase autolysis.

II. Osmotic Phenomena in Plants.

It is evident that water, salts, carbon dioxide and oxygen can, at least occasionally, penetrate plant cells, as otherwise no growth could occur. In case of the higher plants, the same is true of sugars and other bodies.[1] Janse[2] found that so much KNO_3 is absorbed by *Spirogyra* cells in 10 minutes, that it may be easily detected microchemically with diphenylamin-sulphuric acid.

Osterhout[3] grew seeds of *Dianthus barbatus* in distilled water. The rate of growth during the several days of observation was normal. In nature, calcium oxalate crystals are found in the root hairs, but are not formed in the distilled water cultures, showing that the Ca comes from the medium. If placed in calcium solutions, crystals became large enough to see with the polarizing microscope in four hours, showing permeability to Ca.[4]

Nathanson[5] found that nitrates and other substances entered the cell. Ruhland also observed penetration of salts.

Traube-Mengarini and Scala[6] conclude that salts enter plant cells only through the partition walls. At these places there appears an "acid reaction" (bluing of methyl violet). They interpret this as showing that the anion of the salt unites with an H ion of an amino group, forming a free acid, and the kation of the salt unites with the protoplasm. It appears to me that the basis of this conclusion is very slight.

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[1] See Laurent in Livingstone, "The Rôle of Diffusion and Osmotic Pressure in Plants," 1903, p. 67.

[2] *Versl. en Medeel. der Konikl. Akad. van afdeel. Naturs.*, 3. Reeks, IV. part, 1888, p. 333.

[3] *Zeits. f. physik. Chem.*, 1909, LXX., 408.

[4] But compare von Mayenberg, *Jahrb. f. wiss. Bot.*, XXXVI., 381, who found little penetration of salts into fungous hyphæ. And see Demoussy, *Comptes Rendus*, CXXVII., 970.

[5] *Jahrb. wiss. Bot.*, XXXVIII., 284; XXXIX., 601; XL., 403.

[6] *Biochem. Zeit.*, 1909, XVII., 443.

Permeability may be investigated by a study of plasmolysis, which consists in the shrinkage of the surface protoplasm away from the cellulose cell wall, due to the osmotic pressure of the hypertonic solution of a dissolved substance which does not penetrate. A regaining of turgor by the cell while in the hypertonic solution indicates slow penetration of the substance. The plasmolytic method was originated by Nageli, who also noted that a shrinkage resembling plasmolysis but accompanied by outward diffusion of dissolved substances, occurs at death or severe injury to the cell.[1]

The plant cell is surrounded by an elastic cell wall. The internal osmotic pressure may be divided into three resultants: that causing rounding up of the cell is called turgor, that resulting in stretching of the cell wall is sometimes distinguished as turgescence, and that resisting the surface tension of the cell, "central pressure."

The plasmolytic experiments of DeVries[2] and others[3] are interpreted by them as indicating a selective impermeability of the plasma membrane to neutral salts.

In the plasmolytic experiments of Overton[4] all salts plasmolyzed permanently. Non-electrolytes fell in four groups, thus: Cane sugar, dextrose, manit, glycocoll > urea, glucerin > ethylene-alcohol, acetamid > methyl-alcohol, acetonitril, ethyl-alcohol, phenol, aniline, isobutyl-alcohol, isoamyl-alcohol, methyl acetate, ethyl acetate, butyl aldehyde, acetone, acetaldoxim. Diffusion of substances of homologous series increased with molecular weight.

Overton ascertained the permeability of plant cells to alkaloids by their precipitation of the tannic acid in the cell sap. Most alkaloids penetrate rapidly, but only in the form of the free (undissociated) base produced by hydrolysis. Hence the penetration (precipitation and toxic effect) may be prevented by adding a little acid to the medium.

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[1] "Pflanzenphysiol. Untersuchungen," 1885.

[2] *Zeit. physikal. Chem.*, 1888, II., 415; 1889, III., 103.

[3] Cf. Livingstone, "The Rôle of Diffusion and Osmotic Pressure in Plants," Chicago, 1903; Janse, *Bot. Centlb.*, 1887, XXXII., 21; Duggar, *Trans. Acad. Sc. St. Louis*, 1906, XVI., 473.

[4] *Vierteljahrschrift der Naturforschers. Gesell. in Zurich*, XLIV., 88; *Jahr. wiss. Bot.*, 1900, XXXIV., 669.

Pfeffer had shown that methylene blue is precipitated by tannic acid in the cell sap of certain plants.

Some discussion has arisen as to whether the mechanism of the entrance of dyes into plant cells is similar to that of alkaloids. Overton showed that lipoid soluble basic dyes penetrate easily. He at first supposed that only the free color base (undissociated) is able to penetrate the cell.[1] Overton found, however, that triphenylmethane and chinonimid dyes disprove his assumption, showing that it is at least not general. This question was taken up again by Harvey[2] who found that neutral red or methylene blue, which stain *Elodea* leaves in tap water, do not do so if just enough acid be added to the water to prevent any free color base from forming.

He observed that, although these dyes are not precipitated in the cell sap of this plant, they become more concentrated in the cell sap than in the medium. Neutral red is bright red in the cell sap, indicating that the reaction is acid (no free color base is present). He supposes that the absence of any of the dye in the form of the free color base prevents it from diffusing out of the cell, hence it becomes more concentrated within than without.

In using the plasmolytic method, if a cell does not recover from plasmolysis in a solution of a salt, it is said to be impermeable to that salt. However, the cell may recover, but may be killed by penetration of the salt, and shrink again. It is possible that Overton and others failed in some cases to note this transient recovery. Contrary to Overton, Osterhout[3] found *Spirogyra* permeable to alkali-salts and alkaline earth salts, but more easily to Na than to Ca. It is plasmolyzed by .2*M* $CaCl_2$ and not by the isosmotic .29*M* NaCl but by .38*M* NaCl. .195*M* $CaCl_2$ and .375*M* NaCl just failed to plasmolyze. On mixing 100 c.c. .375*M* NaCl with 10 c.c. .195*M* $CaCl_2$, thus decreasing the osmotic pressure of the former, marked plasmolysis occurred. This indicates that Ca decreases the permeability to Na.[1] From further work by the same author, not yet published, it appears that Na increases and Ca decreases the permeability of certain marine plants. Also Fluri[2] obtained increase in permeability by salts of aluminium, yttrium and lanthanum.

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[1] In this connection it is interesting to note that Robertson observed that free color bases, and to a less extent free color acids, are much more soluble in fats than are their salts. This is what we should expect, since the salts dissociate in water, and ions are insoluble in fats.

[2] *Science*, 1910, n.s., XXXII., 565.

[3] *Science*, 1911, n. s., XXXIV., 187; XXXV., 112.

DeVries plasmolyzed cells of *Tradescantia*, containing blue cell sap, with 4 per cent. KNO_3 solution, then added nitric acid until the color changed to red. The acid made the cells permeable to KNO_3 for they regained their turgor and finally burst. This explains the easy penetration of acids into cells. Pfeffer[3] found that if red beet cells, petals of *Pulmonaria*, stamen hairs of *Tradescantia* and other anthocyan-containing cells are placed in extremely dilute HCl or H_2SO_4, they suddenly turn red, indicating immediate penetration of the acid. If allowed to remain but a short time, the cells are not killed, and the color change is reversed on returning the tissues to acid-free water.

I have repeated these experiments, using cells of red beet, red cabbage and red nectar glands of *Vicia faba*, and find that mineral acids penetrate, but that (the lipoid soluble) acetic acid penetrates much more rapidly and also more easily alters the plasma membrane, causing pigment to diffuse out, if not cautiously applied. Alkalis also penetrate, but (the lipoid soluble) ammonia penetrates much more rapidly than the others. Ammonia does not so easily increase the permeability to the pigment as does acetic acid.

Ruhland[4] after staining root hairs of *Trianea*, etc., with the indicators, methyl orange and neutral red, found that mineral acids as well as lipoid soluble acids penetrated.

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[1] The work of Kearney, Report 71, U. S. Dept. of Agriculture, indicates that Ca prevents the plasmolytic and toxic effect of Mg, but this is "false plasmolysis" following death.

[2] *Flora*, 1908, XCIX., 81.

[3] "Osmotische Untersuchungen," Leipzig, 1877, p. 135.

[4] *Jahrb. wiss. Bot.*, 1908, XLVI., 1.

One defect in the plasmolytic method is the fact that the cellulose cell wall, if not very thick, is elastic, and a slightly hypertonic solution may cause the cell to decrease in volume without pressing the protoplasm away from the cell wall. This source of error may be eliminated by substituting calculations of the volume of the cells (as necessary for animal cells) for observations on plasmolysis.

It is well known that movement, and in many cases increase in size of plants is due to changes in turgor of the cells. If we exclude the turgor changes in aerial plants produced by variations in the ratio of the water supply to the transpiration, turgor changes may be due to changes in the osmotic pressure of the external medium, or of the cell sap (due to metabolic changes) or to changes in the permeability of the plasma membrane. Lepeschkin[1] has confirmed Pfeffer in showing that changes in permeability of stipule cells accompany (or immediately precede) changes in turgor. By chemical analysis of the medium he has shown that an outward diffusion of dissolved substances, from the cells, accompanies loss of turgor, and by plasmolytic experiments, that the permeability to certain substances increases.

It is interesting to note the force that may be exerted by such changes in turgor. From measurements of the pull of a stamen hair of *Cynara scolumus* or *Centaurea jacea* on loss of turgor following stimulation, it seems not improbable that the change in turgor amounts to 2–4 atmospheres (Höber). This also indicates the strength of the cell wall necessary to prevent rupture of the plasma membrane. The osmotic pressure of the juices pressed out of plants varies from 3.5–9 atmospheres.[2] The pressing out of the juices causes an error due to chemical changes; on the other hand, in taking the freezing point or pieces of plant tissues, an error arises from lowering of the freezing point by the walls of the capillary spaces. Müller-Thurgau[3] found the Δ (corrected freezing point lowering) of plant tissues = .8–3.1°. Many plants respond to light by definite movements, produced by turgor changes in certain of their cells. Trondle[1] found that light produced changes in permeability of these cells.

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[1] *Ber. deutsch. bot. Gesell.*, XXVI. (*a*), 725.

[2] DeVries, *Pringsheime Jahrbucher wiss. Bot.*, 1884, XIV., 427; Pantanelli, *ibid.*, 1904, XL., 303.

[3] *Landwirtschaftl. Jahrb.*, 1886, XV., 490.

Changes in permeability may not only affect the turgor, but also the assimilation and excretion, and consequently the metabolism and growth of the cells. Chapin[2] observed that CO_2 in certain doses is a stimulant to the growth, not only of green plants but also of moulds. As only a few saprophytes can decompose CO_2, it is not probable that its effect is nutritive. A similar stimulating action of ether and various salts, even such toxic ones as those of zinc, was previously known. These salts probably stimulate without penetrating the cells, since Zn, for instance, is not a constituent of protoplasm.[3] This leads one to suppose that the initial effect of all of these substances is on the surface, changing the permeability of the cells.

Wächter[4] found that potassium decreases the permeability of onion cells. Sugar diffused out of sections of *Allium cepa* placed in distilled water or hypotonic sugar solutions, but a trace of potassium salt entirely prohibited the diffusion. When the K was removed the diffusion recommenced.

Czapek[5] determined increase in permeability by the exosmosis of tannin in cells of *Echeveria* leaves. Various monovalent alcohols and ketones, ether, ethyl urethan, di and tri acetin, Na-oleate, oleic acid, lecithin and cholesterin all just caused exosmosis of tannin in concentrations (aqueous solutions) which had a surface tension of about 0.68. It would appear therefore that these substances, chiefly of the class of indifferent narcotics, alter the cells if they diffuse into them, or diffuse into certain structures such as the cell lipoids or the plasma membrane. It seems more reasonable to suppose that the plasma membrane is the structure affected, and the more the substance lowers the surface tension of water, the more it diffuses into the plasma membrane. When this membrane is altered, it allows escape of tannin. Some substances such as chloral hydrate are effective in less concentration, and probably affect the cell chemically as well as physically.

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[1] *Jahrb. f. wiss. Bot.*, 1910, XLVIII., 171.

[2] *Flora*, 1902, XC., 348.

[3] Cf. Loeb, "Dynamics of Living Matter," pp. 73, 74.

[4] *Jahrb. wiss. Bot.*, 1905, XLI., 165.

[5] "Über eine Methode zur direkten Bestimmung der Oberflächenspannung der Plasmahaut von Pflanzenzellen," Jena, G. Fischer, 1911.

Mineral acids caused exosmosis of tannin when the concentration just exceeded 1/6,400 normal, and the effect is probably due to H ions. At this same concentration Kahlenberg and True[1] found the growth of seedlings of *Lupinus albus* to cease. It appears, therefore, that this cessation of growth is due to increased permeability, causing decreased turgor of the cells.

Changes in permeability may also affect secretion (excretion). The addition or formation of alcohol or acetates causes yeast and other fungi to secrete (excrete) for a short time, various substances, especially enzymes which do not come out in a culture medium lacking the reagent.[2] It appears that the alcohol or acetates increase the permeability of the fungi to these substances.

My own experiments[3] indicate that pure $MgCl_2$ solutions increase the permeability of yeast. A certain per cent. of yeast and dextrose in .3 molecular $MgCl_2$ eliminated CO_2 more rapidly than $.5M$ NaCl or $.325M$ $CaCl_2$, all which have about the same freezing points. Also, the CO_2 elimination was more rapid in the magnesium solution than in a solution of the same concentration of $MgCl_2$ with either of the other salts in addition, or in a solution containing NaCl and $CaCl_2$ in the same concentrations as in their respective pure solutions, or in a solution of all three salts, or in tap or distilled water. In order to determine whether the magnesium entered the cells I took two equal masses of compressed yeast and agitated one in H_2O and the other in a molecular solution of $MgCl_2$ for 5 hours, then washed each rapidly in H_2O by means of the centrifuge. The ash of the magnesium culture = .048 gram, that of the control = .0466 gram. Evidently the Mg did not enter the yeast to any great extent, and probably acted on the surface, increasing the permeability.

Ewart[4] observed that after placing plant tissue in 2 per cent. HCl and washing in water its electric conductivity (ionic permeability) was increased. If one portion of the plant is stimulated, the stimulus may be transmitted to other portions. In this way increase in electric conductivity was produced by stimulation of a point outside the path of the current.

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[1] Kahlenberg and True, *Botanical Gazette*, 1896, XXII., p. 81.

[2] Zangger, "Asher and Spiro's Ergeb. d. Physiol.," 1908, VII., 144.

[3] McClendon, *Am. Jour. Physiol.*, 1910, XXVII., p. 265.

[4] "Protoplasmic Streaming in Plants," Oxford, 1903, p. 96.

Whereas many plants are very sensitive to sudden and extreme changes in osmotic pressure, Osterhout[1] found that certain marine algæ thrived when subjected daily to a change from fresh water, to sea water evaporated down until it crystallized out, and vice versa. He does not state whether these algæ survive extreme plasmolysis or whether they are so easily permeable to salts as not to be plasmolyzed by the saturated sea water or burst by the fresh water.

For regulation to slight changes in the osmotic pressure of the medium, a change in size of the cell altering the turgescence, or tension of the cell wall, is sufficient.

If *Tradescantia* cells are placed in a hypotonic solution, they begin to swell. But soon crystals of calcium oxalate are formed in the cell sap, and in this way the turgor, due chiefly to oxalic acid, is reduced.[2] It would be interesting to know what is the source of the Ca. Was it previously in combination with proteids?

The accommodation to a hypertonic medium takes place, according to van Rysselberghe, partly through absorption of substances of the medium and partly through metabolic production of osmotic substances, chiefly the transformation of starch into oxalic acid.[3]

III. Bio-electrical Phenomena.

1. *In Plants.*

Change in permeability of the plasma membrane to ions would necessarily cause electrical change due to its influence on the migration of ions. These electrical changes actually occur, and may be easily studied.

Stimulation or wounding in plants is accompanied by an electronegative variation of the affected surface. This negative region spreads in all directions over the surface, but the rate of propagation[1] is much slower than the similar process in muscle or nerve.[2]

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[1] Univ. of Cal. Pub., Bot., 1906, II., 227.

[2] Van Rysselberghe, Mém. d. l'Acad. royale de Belgique, 1899, LVIII., 1.

[3] Compare von Mayenberg *Jahrb. f. wiss. Bot.*, XXXVI., 381.

Pfeffer[3] supposed that the plasma membrane is normally permeable to ions of only one sign. Since the normal cell surface is positive in relation to the cell interior (cut surface) we may conclude that the plasma membrane is normally more permeable to kations (less permeable to anions). Just as the negative variation of wounding is due to the removal or rupture of the plasma membrane, so the negative variation of stimulation would, on the membrane hypothesis, be due to increase in permeability of the plasma membrane to the confined anions.

An alternative hypothesis is that these electrical changes result from changes in metabolic activity. The production of an electrolyte whose anion and kation have very different speeds of migration (such as an acid or alkali) would cause electrical changes. But how are we to account for changes in metabolic activity? There exists varied evidence for changes in permeability, and it is simpler to assume that changes in metabolic activity and electrical changes are both the result of changes in permeability.

Kunkel[4] tried to explain the vital electrical phenomena as the result of the movement of fluids in the vessels of the tissues, but bio-electrical changes may occur without such movement of fluids (Burdon-Sanderson).

Kunkel observed in 1882[5] that the movement of the leaf of *Mimosa pudica* is accompanied by an "action current," or negative variation of one surface of the pulvinus. Similar results on *Dionæa* leaves were obtained by Munk[6] and specially studied by Burdon-Sanderson.[7] It was stated above that Lepeschkin had shown that the turgor changes in plants were accompanied or immediately preceded by changes in permeability to certain substances. The electrical phenomena suggest that the turgor change is accompanied (or immediately preceded) by increase in permeability of the plasma membrane to anions. Burdon-Sanderson states that, whereas the movement resulting from turgor change begins 2.5 seconds after stimulation, the negative variation reaches its maximum 1 second after stimulation. This may be due to the mechanical inertia, or the time required for the diffusion of substances.

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[1] Which is in *mimosa* 600–1,000 times as fast as the geotropic impulse in a root.

[2] Fitting, "Asher and Spiro's Ergeb. d. Physiol.," 1906, V., 155.

[3] "Pflanzenphysiologie."

[4] *Arch. f. d. ges. Physiol.*, 1881, XXV., 342.

[5] See Winterstein's "Handbuch der vergleichenden Physiologie," III. (2), 2, p. 214.

[6] *Arch. f. Anat. u. Physiol.*, 1876, XXX., 167.

[7] *Proc. Roy. Soc. London*, 1877, XXV., 441; *Philos. Trans.*, 1888, CLXXIX., 417.

It was stated in the preceding chapter that light changes the permeability of the plasma membrane, and Waller[1] found corresponding electrical changes due to light, but not always in the same direction in different plants. This inconstancy in direction is probably due to the fact that light not only influences the permeability, but also the assimilation, and changes in assimilation produce electric changes. This is supported by the fact that Querton[2] found that assimilation as well as electric change is most affected by the longer light rays.

2. *In Muscle and Nerve.*[3]

Ostwald[4] proposed the hypothesis that the electric phenomena of muscle, nerve and the electric organs of fish (which may reach several hundred volts) are produced with the aid of semipermeable membranes. The alternative theory of Hermann, which would account for the current of injury by assuming the production of some electrolyte (alkali?) in the wounded region, whose anions and kations have very different speeds, seems less probably to be the correct one.

According to the "membrane theory," the muscle or nerve element is surrounded by a semipermeable membrane allowing easier passage to kations than to anions. The kations passing through the membrane are held back by the negative field produced by the confined anions, but owing to their kinetic energy, the kations pass out far enough to give the outside of the cell surface a positive charge. Therefore any portion of the surface that is made freely permeable to anions becomes electronegative in relation to the remainder of the surface. This negative variation may be produced by artificially removing or altering a portion of the membrane (producing the current of injury) or as the result of normal stimulation, making it permeable to anions (action current).

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[1] *Jour. of Physiol.*, 1899–'00, XXV., 18.

[2] "Contribution à l'étude du mode de la production de l'électricité dans etres vivantes," *Travaux de l'Institut Solvay*, 1902, V.

[3] Cf. R. Lillie, *Amer. Jour. Physiol.*, 1911, XXVIII., 197.

[4] *Zeit. physik. Chem.*, 1890, VI., 71.

Bernstein resorted to mathematical proof of this hypothesis. We will not here go into details, but the gist of the matter is that if the process were as we have imagined it, the electromotive force of the current of injury, or action current, should be proportional to the absolute temperature. He found this to be true for temperatures between 0° and 18°, but between 18° and 32° the E.M.F. was found to be too small. The muscle was not permanently injured by exposure to the higher temperatures for the length of time necessary for the experiments. Bernstein explained this discrepancy by the further assumption that at the higher temperatures the plasma membrane became slightly more permeable to anions.[1]

Since the muscle contains a higher per cent. of potassium than the blood plasma or lymph, it might be supposed that K ions passed outward through the plasma membrane and gave the surface of the muscle element the positive charge. But if this were the case, the current of injury should be reversed by placing the muscle in a solution containing potassium in greater concentration than in the muscle. This reversal, however, was shown by Höber not to occur. Since lactic and carbonic acids are produced by muscle and diffuse out in increased amount on contraction, one might suppose H ions to give the muscle surface the positive charge. This is only a guess (and a poor one, since undissociated molecules of CO_2 and lactic acid are lipoid-soluble) but may be convenient until some better one is proposed. Perhaps the carbonic acid combines with amphoteric proteids, which then set free H^+ and HCO_3^- ions, thus increasing the ionization and therefore reducing the number of undissociated molecules, which can escape.[1]

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[1] This is similar to the conclusion reached by Biataszewicz, *Bull. d. l'Acad. d' Sc. d. Cracovie, Sc. Math. e. Nat.*, Oct., 1908, p. 783, in regard to the unfertilized frog's egg. In order to explain his observation that the rate of swelling in tap water increased 5 times for every 10° rise in temperature, he assumed that heat increased the permeability to H_2O. This would seem to be the simplest explanation, provided the swelling were not due to chemical production of osmotic substances: and since the Δ of the ripe ovarian egg is .48° but is reduced to .045° after oviposition, *Biochem. Zeit.*, 1909, XXII., 390, much if not all of the swelling is probably due to the initial osmotic pressure of the egg interior.

Since Osterhout showed that certain electrolytes may alter the permeability of cells, we might expect to find, on the membrane hypothesis, an effect of salts on the electric polarization of muscle. Höber[2] observed that a portion of the surface of a muscle treated with certain salts, KCl for instance, becomes electro-negative (more permeable to anions) whereas a portion treated with NaI or LiCl becomes positive (still less permeable to anions than is the normal unstimulated muscle). The order of effectiveness of the ions is as follows: $Li < Na < Cs < NH_4 < Rb < K$ and $CNS < NO_3 < I < Br < Cl <$ valerianate, butyrate, propionate, acetate, formate $< SO_4$, tartrate. Similar ionic series were found by Overton, R. Lillie, Schwartz, Mathews, Grützner, Höber, and Mayer in the effect of salts on the functional activity of muscle, nerve and cilia, but the exact relation of these phenomena to permeability is not understood in every case. Pure solutions of salts of alkali metals may "inhibit" muscle by *increasing* permeability, but salts of alkali earth metals are said to "inhibit" by *decreasing* permeability. . Mayer says that the effect of salts on cilia is the reverse of that of muscle, but the relation of this to permeability is not known. Since ions affect the aggregation state of hydrophile colloids in the same or exactly reversed order, and the kation series is found in no other known physico-chemical phenomena, it might be supposed that the semipermeable membranes of muscle are colloidal.

It seems probable that sugar solutions inhibit the activity of muscle by increasing the permeability, but since sugar is not an electrolyte this question cannot be tested by electric methods.

A negative variation of muscle may also be produced by the so-called "hæmolytic" substances, but is irreversible, whereas that produced by salts may be reversible. In this connection it is interesting to note that Overton[1] found the permeability of muscle to be similar to that of plant cells.

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[1] Roaf, *Q. J. Exper. Physiol.*, 1910, III., 171, supposed the anion to be protein; however it has not been shown that proteids, or even amino acids diffuse out on stimulation. I do not see that the speculation of Galeotti, *Zeit. f. Allgem. Physiol.*, 1907, VI., 99, is at all explanatory.

[2] *Loc. cit.* and *Pflüger's Arch.*, 1910, CXXXIV., 311.

It might appear to the reader that the membrane theory is merely wild speculation. What proof have we that on injury or during contraction the muscle is more permeable to any ion? DuBois Reymond[2] and Hermann[3] explained the fact that living muscle has a greater electric resistance than dead muscle on the hypothesis that the resistance of living muscle is due to the presence of membranes, which become more permeable at death. They demonstrated the resistance of muscle tissue to the passage of ions by the fact that electric polarization occurs in muscle tissue on the pasage of an electric current. It seems to me that Kodis[4] and Galeotti[5] take a step backward, in attributing the decreased resistance of dead muscle to the liberation of ions. Galeotti tried to support his view by determinations of the freezing points of the living and dead muscle, but found on the contrary that the change in electric conductivity of the muscle did not correspond to the change in the osmotic pressure.

Du Bois Reymond[6] observed that the electric conductivity of muscle changes on (during?) contraction and Galeotti[7] found it to be greater on strong contraction than on weak contraction, and least on fatigue-exhaustion or cold-anæsthesia. However, the duration of a contraction is momentary (about 1/5 second for frog's muscle) and it is not clear that these investigators measured the conductivity accurately during such a brief period, in fact they probably measured it after contraction. Therefore I decided to repeat these experiments, using a method by which I could measure the conductivity during the actual contraction period, as well as in the unstimulated condition.[8]

[1] *Pflüger's Arch.*, 1902, XCII., 115.

[2] "Untersuchungen über thierische Electricität," 1849.

[3] *Pflüger's Arch.*, 1872, V., 223, VI., 313.

[4] *Am. Jour. Physiol.*, 1901, V., 267.

[5] *Zeit. f. Biol.*, n. f., 1902, XXV., 289; 1903, XXVII., 65.

[6] *Loc. cit.*

[7] *Loc. cit.*

[8] McClendon, *American Journal of Physiology*, 1912, XXIX., 302.

Experimental.

Platinum electrodes, platinized with platinic chloride containing a little lead acetate, and of a form similar to those designed by Galeotti, were used. Galeotti stimulated the muscle through the same electrodes used in measuring the electric conductivity, by switching on a different electric current. Though it were possible to throw a switch quickly enough to have the current for measurement of conductivity pass through the muscle during contraction, it would be necessary to use a string galvanometer to take the reading, and this method would probably not be very accurate. A more accurate method is that of Kohlrausch, in which a rapidly alternating current reduces polarization at the electrodes and in the tissue, but it is necessary to throw the muscle into tetanus in order to have time for the reading. I accomplished this by using the same current for stimulation and measurement of conductivity. A very small induction coil was fitted with a rheostat in the primary. Another rheostat in the secondary could be thrown out of the circuit by a switch. By adjusting the rheostats, a current strong enough to be distinctly heard in the telephone, yet too weak to stimulate the muscle, was obtained. By switching the resistance out of the secondary circuit, the current could immediately be increased so as to throw the muscle into tetanus. Since the Wheatstone bridge was used, the difference in current strengths had no direct effect on the readings. The conductivity increased from 6 to 28 per cent. (being usually about 15 per cent.) on stimulation.

We have, then, evidence for the increase in permeability of muscle to ions during contraction, but what relation has this to the mechanism of the contractile process? It has been suggested by D'Arsonval, Quincke, Imbert, Bernstein, Galeotti and others that the increased permeability to ions causes a disappearance of the normal electrical polarization of the elements, whose surface tension consequently increases, causing them to round up (shorten). But what are the elements concerned? It would be confusing to assume them to be the fibers, as then the function of the complicated internal structure would be unexplained. They are probably not the sarcous elements (portions of fiber between 2 Z-lines) as the rounding up of these elements would elongate the muscle. And even though contraction were produced by inequality in surface tension, as assumed by Macallum[1] the total surface change would be so small as not to account for the energy liberated in contraction. In order to avoid this last difficulty Bernstein made use of hypothetical ellipsoids. These were surrounded by elastic material to account for elongation of the muscle.[2]

The great differences of potential (several hundred volts) that may be produced by the electric organs of fish, is achieved by the arrangement of the modified muscle plates in series. All of the plates have the nerve termination on the same side. On stimulation of the nerve, each plate becomes negative, first on the nerve termination side, and thus the negative side of one plate touches the positive side of the next plate. In this way the direction of the current may be determined by studying the anatomy of the innervation. This rule, discovered by Pacini, finds an exception only in *Malopterurus*, whose electric organ is supposed by Fritsch to be derived, not from muscle but from skin glands.

The electric fish are *relatively* immune to electric currents passed through the medium. This is not merely an apparent immunity due to the fish being out of the path of the current, or the current being short circuited by sea water (in case of marine fish). I have received severe shocks from a torpedo that was entirely submerged in sea water.

3. *Amœboid Movement.*[3]

The normal unstimulated surface of plant and animal tissues is electro-positive in relation to the cut or injured surface of the cells. We have given reasons for assuming that this indicates greater permeability of the plasma membrane to kations than to anions, the latter accumulating in the cell interior, gives it a negative charge.

There are two reasons for believing that this is true also of the *Amœba:*

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[1] *Science*, n. s., 1910, XXXII., 822.

[2] Meigs., *Am. Jour. Physiol.*, 1910, XXVI., 191, supposes the rounding up of muscle elements due to increased turgor.

[3] McClendon, *Arch. f. d. ges. Physiol.*, 1911, CXL., 271.

1. If a weak electric current is passed through water in which an *Amœba* is suspended, it is carried passively toward the anode, indicating that it has a negative charge. This charge may be due to confined anions.

2. If a stronger electric current is passed through an *Amœba*, it begins to disintegrate first at that surface nearest the anode. The disintegration is probably due to the accumulation of ions retarded by the plasma membrane. The ions in the medium are free to pass around the *Amœba*, but the contained ions must pass the plasma membrane in order to migrate to the electrodes. Since the disintegration is toward the anode, it is probably due to anions which cannot get out of the *Amœba*. Since no corresponding disintegration begins toward the kathode, the plasma membrane is probably more permeable to kations.

The surface tension of the *Amœba* is very low, and apparently increases on strong stimulation (indicated by rounding up of the *Amœba*). We saw that stimulation in plant and muscle cells caused increased permeability to ions, and consequently disappearance of the normal electrical polarization, and thereby causing increased surface tension. We might conclude therefore that the low surface tension of the *Amœba* is caused by electric polarization, due to the production of some metabolic electrolyte whose anions cannot escape; and that strong stimulation causes increased permeability and hence disappearance of the electrical polarization.

This would explain all negative tropisms of the *Amœba*. The surface tension of the portion most strongly stimulated is increased, and the *Amœba* flows away from the stimulus.

In order to explain positive tropisms we would have to make another assumption. If the stimulus did not act directly on the plasma membrane, but penetrated the *Amœba* and acted on the protoplasm, and increased the production of the metabolic product producing polarization of the plasma membrane, it would thereby decrease the surface tension. The local decrease in surface tension would cause the *Amœba* to flow toward the source of the stimulus, just as the quicksilver drop in dilute HNO_3 flows toward potassium bichromate in Bernstein's experiment.

All stimuli producing positive tropism would then have to penetrate to a greater or less distance into the *Amœba*. But the same stimulus thus acting on the interior might, in greater intensity, affect also the plasma membrane, increasing its permeability and changing the positive to negative tropism. Such a change of the sign of tropism has been observed.

Soap lowers the surface tension of fats and lipoids, and Quincke, Bütschli, Loeb, Robertson and others supposed that lowering of the surface tension of living cells might be due to soap. However, I found that soap always causes negative tropism in *Amœba*, probably because it increases the permeability of the plasma membrane.

4. *The Propagation of the Bio-electric Changes.*

On the hypothesis, that the electric phenomena in muscle and nerve, as well as other animal and also plant tissues, is due to change in permeability to ions, we might hope to explain the wave-like propagation of these changes. Since extraneous electric currents "stimulate" all tissues (presumably by increasing permeability) thus causing them to produce additional electric phenomena, it seems natural that these latter would be self-propagating. It is probably the negative variation of nerve which stimulates the muscle, and the negative variation of the portion of the muscle fiber adjoining the nerve ending, which stimulates the adjacent portions of the muscle. Nernst found mathematical proof that electric stimulation is due to change in ionic concentration at the semipermeable membranes.

I have found evidence that the negative variation (current of injury) in plants, may strongly affect adjacent cells. If an electric current of suitable density is passed through plant or animal tissue, negatively charged colloids in the protoplasm migrate toward the anode. I have observed this movement in living cells, and the resulting displaced bodies in histological sections. In certain cases there may be some doubt whether the colloids moved toward the anode, or water toward the kathode, but in others, easily distinguishable bodies such as chromatin granules or threads moved toward the anode.

If the tip of a root be cut off we observe a negative variation of the cut surface. This produces an electric current through the medium and surrounding tissue. The fact that the current actually passes through adjacent cells is shown by a displacement of their contained colloids, identical in appearance with the displacement produced by the currents used in the above experiments. Nêmec[1] apparently observed these changes but did not correctly describe or interpret them.

The fact that an electric current on increase (make) stimulates muscle at the kathode, and the fact that the muscle surface is normally positive in relation to the interior (cut surface), probably indicates that stimulation is produced by a rapid depolarization of the muscle surface.

If this reasoning be applied to an individual contractile element, we may assume that the current causes kations to leave the outer surface of the membrane, and other kations to be attracted toward the inner side of the membrane, and thus the polarization disappears or may even be reversed. Just how this causes an increase in permeability of the membrane is a matter which we will leave to the future for discussion.

It has been supposed that the stimulated region acts as kathode to the adjacent portions, and these in turn act as kathodes to the next portions and so the stimulus is propagated.

Stimulation of a part of the surface, causing it to become more permeable to ions, depolarizes the adjacent parts of the surface owing to the fact that confined anions migrate through the permeable region and neutralize the charges of the kations on adjacent parts of the impermeable region (see Fig. 1). For this reason the increase in permeability is propagated.

This explanation of the phenomenon in a single element holds for a tissue made up of many elements provided these are in contact, as illustrated by the accompanying Fig. 2. This is probably the mechanism of propagation of the negative variation (and "stimulus") in many plant and animal tissues.

This mechanism accounts for the movement of the negative variation over a surface. But it may be possible for this electric change to jump from one element, to another not touching it. The observations on the current of injury, cited above, show that increased permeability of part of a tissue surface, may cause electric currents to flow through cells some distance from the wound. These currents probably stimulate the cells through which they pass, which in turn become permeable and produce electric currents. This explains the propagation of stimuli through loose tissues, and the structural changes, as observed by Nêmec.

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[1] "Reizleitung u .d. reizleitenden Strukturen b. d. Pflanzen," Jena, 1901.

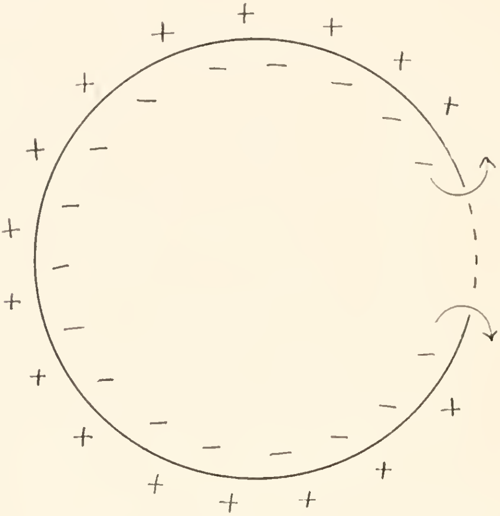

Fig. 1.

Anions represented by minus sign, kations represented by plus sign. Arrows denote the direction of migration of ions. The large circle represents the plasma membrane, the dotted line denoting the permeable and the continuous line, the impermeable portion.

The rate of propagation of the "wound stimulus" is very slow, whereas that of propagation of the "stimulus" (negative variation) in sensitive plants is more rapid, and that of the nerve impulse still more rapid. We have not, however, sufficient data to show whether this is a mathematical objection to the hypothesis.

The streaming movements in plants may be stopped by a strong stimulus or "shock." This stimulus is usually propagated in one or more directions. Ewart[1] states that the rate of propagation at 18° in a single elongated cell of *Nitella* is 1–20 mm.

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[1] *Loc. cit.*

per sec., but where it has to pass cell walls .001–.03 mm. per sec. However, the stoppage of the streaming was his criterion of the presence of the stimulus, and probably the banking of the stream at one point, soon stopped the whole stream thus simulating the propagation of the stimulus.

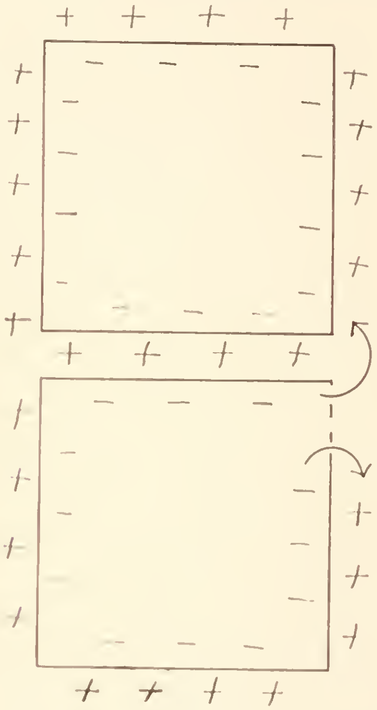

FIG. 2.

The squares represent the plasma membranes of adjacent cells. For further explanation see Fig. 1.

IV. NARCOSIS.

If stimulation consists in increase in permeability, we should expect anæsthetics to prevent this change. The object of this chapter is to present evidence that may support or refute such a hypothesis.

Overton observed that warm- and cold-blooded vertebrates, insects and entomostraca, require practically the same concentration of the anæsthetic for narcosis. Certain groups of worms require double, and protozoa and plants six times this concentration. We might conclude from this that nerves (and especially medullated nerves?) are more susceptible to narcosis than are other cells. All groups of worms contain nerves, but Loeb has shown that certain worms may perform coördinated movements after the nerves are cut, hence the higher concentration of the narcotic required to quiet them. However it should be remembered that *over-stimulation* causes rounding up and quiescence of *Amœba* and muscle may be paralyzed by increasing the permeability. The growth of plants is increased by a certain concentration of ether and retarded by a greater concentration. It may be that true narcosis (decreased permeability) of protozoa and plants cannot be produced by such substances as ether, etc.

Vertebrate nerve tissues are rich in lipoids (which have similar solubilities to neutral fats) and it is therefore significant that Overton and also Meyer[1] found that the partition coefficient of anæsthetic between olive oil and water corresponds to its anæsthetic power. Meyer[2] showed further, that with change of temperature, the change in the partition coefficient between oil and water, and the anæsthetic power of the substance were parallel. Pohl, Frantz, Gréhaut, and Archangelsky found that chloroform, ether, alcohol, chloral-hydrate or acetone, became more concentrated in the central nervous system than in other tissues. This is probably due to the absorption of the narcotic by the lipoids (especially the immense mass of myelin) in the nerve tissues.

If it could be proven that the plasma membrane consists of lipoids, this solubility of narcotics might be considered direct evidence for or against the permeability hypothesis, but lacking such proof we must first attack the subject from another side.

Höber[3] observed that ethyl-methane, phenyl-methane, chloral-hydrate, chloroform and hypnon, in *low concentration* prevent the production by salts, of the current of injury on muscle. He showed that in *lethal doses* on the contrary these narcotics do not prevent but even *produce* a current of injury, in this way explaining data which might otherwise seem to contradict the first statement. Galeotti and Cristina[1] observed that ether, ethyl-chlorid, and chloroform produce a current of injury on frog's muscle.

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[1] *Arch. exp. Path. u. Pharm.*, 1889, XLII., 109.

[2] *Arch. exp. Path. u. Pharm.*, 1901, XLVI., 338.

[3] *Pflüger's Arch.*, 1907, CXX., 492, 501, 508. Cf. R. Lillie, *Am. Jour. Physiol.*, 1912, XXIX., 373.

We may conclude, then, that anæsthetics, in the concentration producing narcosis, so change the plasma membrane as to prevent salts from making it permeable to anions. This is probably also true of nerve, since Höber found that ethyl-methane in low concentration prevented the sensitizing of nerve with K_2SO_4.

Höber has attempted to connect these facts with the lipoid solubility of narcotics. Moore and Roaf[2] had observed that *small quantities* of such narcotics as chloroform, alcohol, ether, or benzol, precipitated lipoids extracted from organs and suspended in water. But Höber and Gordon[3] found that colloidal solutions of lecithin were not precipitated, but were made transparent by ether or chloroform in *high concentration*. Similarly, Goldschmidt and Pribram[4] observed that lecithin suspended in NaCl solution, which is dissolved by chloral hydrate, methane, or cocaine, in high concentration, is precipitated by them in low concentration. On the other hand, Koch and McLean[5] state that chloral, hypnon, acetone, or pure ether, do not change the size of colloidal particles of lecithin (*i. e.*, make them easier or more difficult to salt out). Calugareanu[6] explains the mechanism of the precipitation of lipoids by anæsthetics by the increase in size of the particles due to absorption of the anæsthetic.

Thus there seems to be a parallel difference between the action of low and high concentrations of anæsthetics on muscle and nerve, and the action of the same on lipoid suspensions, but this does not hold true for all cases. Moore and Roaf[7] conclude that anæsthetics are bound, not only by lipoids, but also by proteids, and their charactersitic action on the permeability of the living cell may be due to their action on proteids. In other words, the plasma membrane may be entirely proteid.

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[1] *Arch. allg. Physiol.*, 1910, X., 1.

[2] *Proc. Roy. Soc. London*, 1904, LXXIII., 382; 1906, LXXVII., 86.

[3] *Hofmeisters Beitrage*, 1904, V., 432.

[4] *Zeit. f. exper. Path. u. Ther.*, 1909, VI., 1.

[5] *Jour. Pharm. and Exp. Ther.*, 1910, II., 249.

[6] *Biochem. Zeit.*, 1910, XXIX., 96.

[7] *Loc. cit.*

It is well known that during narcosis little or no oxygen is absorbed by nerve tissue. Verworn and his pupils assumed that the narcotic directly suppressed oxidation. On the other hand Mansfeld[1] supposed that the narcotic dissolving in a lipoid plasma membrane made it less permeable to oxygen. It would be more in harmony with the phenomena considered in previous chapters, to suppose that the narcotic in low concentration decreased the permeability of the plasma membrane to the anions and molecules of some acid end product of oxidation, and thus stopped the combustion. An objection to this hypothesis is made by Warburg[2] who found that phenylurethan, which only slightly reduces oxidation in certain cells, fertilized eggs, delayed cell division enormously. With greater concentration of the narcotic, oxidation was greatly reduced.

V. Osmotic Properties of the Blood Corpuscles.

Hamburger and Bubonavik[3] have concluded that the erythrocytes are permeable to K, Na, Ca and Mg. However, the opposite conclusion was reached by previous workers.

Gyrn's,[4] Hedin,[5] Traube[6] and others observed that the erythrocytes are relatively impermeable to neutral salts (exc. NH_4 salts) amino acids, various sugars and hexite, slowly permeable to erythrite, more permeable to glycerine, and easily permeable to monovalent alcohols, aldehydes, ketones, esters, ether, and urea. In general, it may be said that the erythrocyte is permeable to lipoid-soluble substances or those that lower the surface tension of water. Such substances (for instance, ether) become more concentrated in the corpuscle than in the serum. Saponin becomes 120, and ammonia 880 times more concentrated in corpuscle than in serum.[7]

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[1] *Pflüger's Arch.*, 1909, CXXIX., 69.

[2] *Zeit. physiol. Chem.*, LXVI., 305.

[3] *Arch. internat. de Physiol.*, 1910, X., 1.

[4] *Pflüger's Arch.*, 1896, LXIII., 86, and *Koninkl. Akad. von Wetensch. Amsterdam*, 1910, p. 347.

[5] *Pflüger's Arch.*, 1897, LXVIII., 229; 1898, LXX., 525.

[6] *Biochem. Zeit.*, 1908, X., 371.

[7] Arrhenius, *Biochem. Zeit.*, 1908, XI., 161.

The erythrocytes are practically impermeable to ions. Stewart[1] observed that they offered a great resistance to the electric current. It is difficult to remove all of the serum from a mass of erythrocytes, but Bugarsky and Tangl, working independently of Stewart, obtained sediments of corpuscles having a conductivity of only 1/50 that of the serum. This indicates that the corpuscles are practically impermeable to both classes of ions, for if permeable to ions of one sign, they would probably not be such good insulators. The electric conductivity of the ash (made up to equal volume) of the corpuscles is about that of the serum, although the osmotic pressure of the solution of ash of the latter is greater.[2]

Hence an increase in electric conductivity of the corpuscles (as will be considered below) indicates increased permeability to ions. After the corpuscle becomes permeable to ions, further increase in conductivity might be due to liberation of ions from combinations with colloids in the interior. However many ions, for instance PO_4, cannot be liberated without incineration or other rigorous treatment. Increase in conductivity of the blood by laking agents has been proven to be chiefly due to increased permeability of the corpuscles, since the conductivity of the serum never shows so great an increase on the addition of the laking agent, and is usually diminished (by the hæmoglobin) if the corpuscles are present.

The portion of the normal corpuscle presenting the greatest resistance to the electric current is the surface layer, since Höber[3] observed that the conductivity of the interior of the corpuscle (determined by its dielectric value) is many times greater than that of the corpuscle as a whole. Peskind[4] caused bubbles of nitrogen to form within the corpuscle and observed that they were retained by a superficial membrane. This may be the membrane which resists the electric current.

The chemical composition of the corpuscle is supposed to bear some relation to its permeability. Aside from the hæmoglobin, and the rather low water content (60 per cent.) the corpuscle is composed of lecithin and cholesterin with a little nucleo-proteid. It is probable that these lipoids are chemically different in different species of animals, since Lefmann[1] observed that the lipoids of erythrocytes of the same species are not toxic, whereas those of another species may be very toxic.

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[1] *Science*, Jan. 22, 1897.

[2] Moore and Roaf, *Biochem. Jour.*, III., 155.

[3] *Pflüger's Arch.*, 1910, CXXXIII., 237.

[4] *Am. Jour. Physiol.*, VIII.

The distribution of these substances in the corpuscle has not been ascertained Pascucci[2] supposed the corpuscle to be a bag of proteid impregnated with lecithin and cholesterin and filled with hæmoglobin. He found that artificial lecithin-cholesterin membranes were made more permeable to hæmoglobin by the laking agents, saponin, solanin and tetanus or cobra poison. Dantwitz and Landsteiner suppose the lecithin to be in combination with protein.

Hoppe-Seyler assumed the hæmoglobin to be in combination with lecithin in the corpuscle, and Bang[3] has shown that lipoids may be fixed by hæmoglobin. It seems evident that there does not exist an aqueous solution of hæmoglobin within the corpuscle, since hæmoglobin crystals may be made to form in *Necturus* corpuscles without extraction of water. Furthermore, Traube and Goldenthal[4] find that hæmoglobin has a hæmolytic action, and unless there exists some body within the corpuscle which antagonizes this action (as serum does) a hæmoglobin solution could not be retained by the corpuscle. Probably all of the so-called "stroma" constituents, not in combination with the hæmoglobin, form the plasma membrane of the corpuscle.

Under certain conditions, the hæmoglobin comes out of the corpuscles, and the blood is said to be laked. Laking of "fixed" corpuscles occurs only after the removal of the fixing reagent. Thus, sublimate-fixed corpuscles may be laked by substances which combine with mercury, such as potassium iodide, sodium hyposulphite or even serum proteids. The fact that they may be laked by heating in water is probably because the nucleo-histone is not fixed by sublimate. This process is prevented by hypertonic NaCl solution, presumably on account of its power to precipitate nucleo-histone (Stewart). Formaldehyde-fixed corpuscles may be laked by ammoniacal water, at a temperature which must be higher, the more thoroughly they have been fixed. Ammonia combines with formaldehyde.

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[1] *Beiträge chem. Physiol. u. Path.*, XI., 255.

[2] *Hofmeister's Beiträge*, 1905, VI., 543, 552.

[3] *Ergeb. d. Physiol.*, 1907, VI., 152.

[4] *Biochem. Zeit.*, 1908, X., 390.

Stewart[1] supposes that the hæmoglobin must be liberated from some compound before the blood can be laked. We cannot say that the corpuscle is always permeable to hæmoglobin from within outward. However the corpuscle probably is impermeable to it from without inward, since it does not take up hæmoglobin from a solution, and after the blood is laked the serum contains hæmoglobin in greater concentration than the "ghosts" do.

At any rate, permeability to hæmoglobin appears to be independent of permeability to salts, since Rollett[2] found that laking by condenser discharges may set free the hæmoglobin without the corpuscle becoming permeable to ions. Stewart[3] concluded that the same is true of laking with sodium taurocholate (even after considering the depressing action of hæmoglobin on the conductivity).

Stewart[4] and others had already shown that blood laked by minimal applications of such laking agents as freezing and thawing, heating (to 60°), foreign serum, and autolysis (spontaneous laking) cause but a slight increase in the permeability to ions, whereas the continued application of some of these agents, or especially such violent reagents as distilled water and saponin, cause a marked increase in electric conductivity. On the other hand, if saponin is added to defibrinated blood at 0°, the conductivity of the corpuscles to ions begins to increase before any hæmoglobin escapes from the corpuscles.

The liberation of the hæmoglobin by some laking agents may be due to the direct action of the reagent in breaking up the compound in which the blood pigment exists, but is probably sometimes a secondary effect, following increase in permeability to electrolytes.

It has been shown that many laking agents, lipoid solvents, saponin unsaturated fatty acids, soaps, and hæmolysins (containing lipase) are such as would alter lipoids physically or chemically, whereas pressure, trituration, shaking, heat, condensor discharges, freezing and thawing, water, drying and moistening, salts (including bile salts), acids and alkalis, might act also on proteids.

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[1] *Jour. Pharm. and Exper. Therapeutics*, 1909, I., 49.

[2] *Pflüger's Arch.*, 1900, LXXXII., 199.

[3] *Am. Jour. Physiol.*, X.

[4] *Jour. Physiol.*, 1899, XXIV., 211.

Since any treatment which causes great swelling[1] of the corpuscle leads to loss of hæmoglobin, it is probable that stretching or breaking of the surface film increases its permeability. But laking may occur without swelling, and even crenated corpuscles may be laked by sodium taurocholate.

Höber[2] observed that the relative action of ions in favoring hæmolysis is: salicylate $>$ benzoate $> I > NO_3$, $Br > Cl > SO_4$ and $K > Rb > Cs > Na$, Li. Since this is the order in which they affect the aggregation state of colloids, their action is probably on the aggregation state of the colloids of the corpuscle (proteids or lipoids or their combinations).

The permeability of formaldehyde-fixed corpuscles to ions, is greatly increased by extraction of the lipoids with ether, or by treatment with substances such as saponin, which act on lipoids. Since the proteids have been thoroughly fixed, it is evident that they play no part in this process, though they may do so in the non-fixed corpuscles.

The relation of lipoids outside of the corpuscles to hæmolysis has been extensively investigated, and cannot be fully treated here. Willstätter found that cholesterin combines with one of the saponins, destroying its hæmolytic power. Iscovesco[3] concludes that cholesterin combines with soap, and prevents its toxic action.

Changes in permeability of the corpuscles to ions were studied chemically before the application of the electrolytic method. Hamburger[4] and Limbeck[5] observed that when CO_2 is passed through blood, chlorine passes from serum into corpuscles and the alkalescence of the serum is increased. On the other hand, the distribution of sodium and potassium is not changed.[6]

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[1] Roaf, *Q. J. Exper. Physiol.*, III., 75, supposes this swelling to be due to ionization and hence increased osmotic pressure of hæmoglobin.

[2] *Biochem. Zeit.*, 1908, XIV., 209, and *loc. cit.*

[3] *Comptes Rendus, Soc. Biol.*, 1910, LXIX., 566.

[4] *Zeit. f. Biol.*, 1891, XXVIII., 405.

[5] *Arch. exp. Path.*, 1895, XXXV., 309.

[6] Gürber, *Sitzungsber. physik.-med. Ges. Wurzburg*, 1895.

Koeppe[1] and Höber[2] explain this process in the following manner: The lipoid-soluble CO_2 enters the corpuscle, and by reacting with alkali albuminates in the protoplasm, gives off more anions than it does in the serum. During the presence of CO_2, the corpuscle is permeable to anions, and the $CO_3^{=}$ or HCO_3^{-} ions pass back into the serum, being exchanged for Cl^{-} ions to equalize the electrical potential. Sodium bicarbonate being more alkalescent than sodium chloride, the titratable alkalinity of the serum is increased.

This explanation is supported by the following facts: When CO_2 is passed through a suspension of erythrocytes in cane sugar solution the latter does not become alkaline. If CO_2 is passed through a mass of centrifuged erythocytes, which are then added to physiological salt solution, the latter becomes more alkaline than the serum in Hamburger's experiment. Any sodium salt may be substituted for serum, and its anions will pass into the corpuscles.[3] Also the number of ionic valences passing into the corpuscle is constant, *i. e.*, if sulphate is used only half as many ions enter the corpuscles as when chloride or nitrate is used. The process is reversed by removal of the CO_2.

This same phenomenon has been observed in leucocytes by van der Schroeff.

There seems to be some relation between hæmolysis and agglutination of the corpuscles. Arrhenius[4] supposed that agglutination by acids is due to the coagulation of the proteids of the envelope. However, since agglutination is followed by precipitation, it seems probable that the loss of the negative electric charge which tends to keep the corpuscle in suspension and causes it to repel every other corpuscle, is partly responsible for the phenomena.

The fact that water-laking is preceded by agglutination might be explained if we assume that increase in permeability to ions leads to loss of electric charge. The charge may be due to the charges on the colloids of the corpuscle or to semi-permeability to ions. The corpuscle is very poorly permeable to ions, but may be slightly more permeable to some one ion than to others. If this ion were more concentrated in the plasma or in the corpuscle, the latter would become electrically charged, and a general increase in ionic permeability would lead to a reduction or loss of this charge. The loss of charge would favor their coming in contact with one another and their precipitation, but their cohesion is probably due to some other change, possibly the exit of adhesive substances, on increase in permeability.

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[1] *Pflüger's Arch.*, 1897, LXVII., 189.

[2] *Pflüger's Arch.*, 1904, CII., 196.

[3] Hamburger and van Lier, *Engelmann's Arch.*, 1902, 492.

[4] *Biochem. Zeit.*, 1907, VI., 358.

VI. Absorption and Secretion.

1. *Absorption through the Gut.*

If a live vertebrate intestine be filled with one portion of a physiological NaCl solution, and suspended in another portion of the same solution, fluid will pass through the wall of the gut from within outward. Cohnheim[1] found that holothurian gut behaves in the same way toward sea water, and the absorption stops if the gut is injured with chloroform or sodium fluoride.

It might be supposed that the hydrostatic pressure produced by the contraction of the musculature, is the driving force of absorption, but on the contrary, Reid[2] found that the wall of the rabbit's intestine behaved in the same way when used as a diaphragm.

Salt is absorbed by an intestine filled with a very hypotonic solution of it, and water may be absorbed when the solution is very hypertonic.

Blood salts enter the intestine when it is injured by an extremely hypertonic solution, or sodium fluoride, chinin or arsenic.

Grape sugar and sodium iodide may pass from without inwards through the wall of a normal holothurian intestine.

Traube[3] claims that absorption is explained by his observation that the surface tension of the contents of the gut is less than that of the blood, but this does not apply to the experiments in which an identical solution was placed on each surface of the wall of the gut. Traube[4] found that the addition of a substance lowering the surface tension increased the absorption of NaCl by the gut.

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[1] *Zeit. physiol. Chem.*, 1901, XXXIII., 9.

[2] *Jour. Physiol.*, 1901, XXVI., 436.

[3] *Pflüger's Arch.*, 1904, CV., 559. Cf. Iscovesco, *Comptes Rendus, Soc. Biol.*, 1911, LXXI., 637.

[4] *Biochem. Zeit.*, 1910, XXIV., 323.

Absorption is probably due to irreciprocal permeability of the wall of the gut. Hamburger showed that dead gut and even artificial membranes showed irreciprocal permeability to certain substances. These artificial membranes were of different composition on their opposite surfaces (parchment paper-chrome albumin, or parchment paper-collodion) and he assumed that the wall of the gut is composed of two osmotically different layers. In reality there may be more than two such layers, and the plasma membranes of the individual cells of the gut may show irreciprocal permeability.

Traube[1] showed that the rate of absorption of a substance by living gut is usually greater the more it lowers the surface tension of water. The order of ions is: $Cl > Br > I > NO_3 > SO_4$, HPO_4 and $K, Na > Ca, Mg$. The order of non-electrolytes, according to Katzenellenbogen[2] is: glycocoll < urea < acetone, mannit < erythrite < glycerine < acetamid, methylalcohol, propylalcohol, amylalcohol.

The rate of absorption through dead ox gut according to Hedin[3] is: $Br > NO_3 > Cl > SO_4$ and $K > Rb > Na > Li > Mg$ and mannit < erythrite < glycerine < urethan < glycocoll < amylenhydrate < glycol < urea < propylalcohol < isobutylalcohol < methylalcohol, ethylalcohol.

The action of poisons on absorption may be due to the alteration of the plasma membranes of the individual cells. Mayerhofer and Stein[4] state that even sugar in certain concentrations increased the permeability of the gut.

2. *Osmotic Relation of Aquatic Animals to the Medium.*

Fredericq found that the salt content of the body fluids of marine invertebrates is about the same as that of sea water. Henri and Lalou[5] showed that the osmotic exchange between cœlom fluid of sea urchins and holothurians and medium is chiefly water. If the sea water was diluted with ¼ vol. of isotonic cane sugar solution, the salt content of the cœlom fluid is very little lowered in 4 hours, and only traces of sugar appear in it. The result is the same with isotonic urea (which easily penetrates most plasma membranes). But the salt content of the blood of elasmobranchs and teleosts is about half that of the sea.

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[1] *Pflüger's Arch.*, CXXXII.

[2] *Pflüger's Arch.*, CXIV., 522.

[3] *Pflüger's Arch.*, 1899, LXXVIII., 205.

[4] *Biochem. Zeit.*, 1910, XXVII., 376.

[5] Winterstein, II. (2), 2.

Botazzi and his colleagues observed that the osmotic pressure of the blood of elasmobranchs is about equal to that of the medium, the salts in the blood being supplemented by organic substances, chiefly urea, of which there is 2–3 per cent.

If elasmobranchs are placed in concentrated sea water, the osmotic pressure of the blood rises, but the ratio of urea to salts remains the same. G. G. Scott found that changes in the density and osmotic pressure of the blood of elasmobranchs accompany changes in the salt content of the medium.

However, in marine teleosts as well as all fresh-water animals which have been studied in this respect, both salinity and osmotic pressure of the body fluids are very different from that of the medium.

The osmotic pressure of the blood of marine teleosts is about half that of the sea, but in fresh-water teleosts it is still less (but much greater than the fresh water). This indicates that there must be a change in the osmotic pressure of the blood as the fish ascends a river. Greene[1] observed that it took salmon 30–40 days to pass the brackish water, in which time they were acclimatized to fresh water. After being in fresh water 8–12 weeks, the osmotic pressure of the blood was reduced only 17.6 per cent. This reduction may be partly accounted for by the absorption of the osmotic substances in the blood by the sexual glands. In harmony with this view is the fact that the osmotic pressure of the blood of the female was reduced much more than that of the male. One salmon, that was very weak and probably dying, showed 32 per cent. decrease in Δ of blood. Sumner[2] observed that changes in weight and salt content of marine teleosts accompany, but are not proportional to changes in the medium.

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[1] U. S. B. F., 1904, XXIV., 445; 1909, XXIX., 129; *Jour. Exp. Zoöl.*, 1910, IX.

[2] Bull. U. S. B. F., 1905, XXV., 53, and *Am. Jour. Physiol.*, 1907, XIX., 61.

Overton observed that if the cloaca and mouth of a frog in fresh water are closed, the frog constantly increases in weight. This can be prevented by the addition of .7 per cent. NaCl to the medium. In a hypotonic solution water is constantly absorbed by the skin and excreted by the kidneys. Fischer's[1] experiment, in which ligature of the leg of a frog caused great swelling below the ligature is probably to be explained by the fact that water was absorbed by the skin but could not reach the kidneys, since the blood circulation was stopped. In regard to Fischer's explanation, compare the results of Sidbury and Gies.[2] Sumner concluded that in the fish, the gills are the chief seat of osmotic exchange.

It appears, therefore, that osmosis occurs through the integument (including gills), kidneys and gut simultaneously, and since the contents of the gut and kidney tubules are not the same as the medium, we should not expect an osmotic equilibrium between the body fluids and the medium. Furthermore, all three of these membranes may show irreciprocal permeability.

Fresh-water fish and non-migratory marine fish are killed by great changes in the medium, even though it be very gradual.

Bert maintained that if fresh-water fish are placed in sea water, the gill capillaries contract and become blocked by the distorted corpuscles. In naked-skinned fishes, not only the gills are affected, but water may be lost from the tissues.

Bert and Sumner both agree that the salts in sea water cannot be replaced by any other substance, without causing the death of certain marine fishes. Mosso[3] claimed that when sharks are placed in fresh water, the gill capillaries become so blocked with laked corpuscles that physiological salt solution could not be forced through them. He observed that the differences in the resistance of certain fish to changes in the salt content of the medium, corresponded to differences in the resistance of their blood cells to the hæmolytic action of such changes. Sumner,[4] however, states that this blocking of gill capillaries does not occur in sharks or marine teleosts in fresh water.

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[1] Fischer, M. H., "Œdema," J. Wiley & Sons, 1910.

[2] *Soc. Exper. Bio. and Medicine*, 1911, VII., 104.

[3] *Biol. Centlb.*, 1890, X., 570.

[4] *Proc. Seventh Internat. Zool. Congress*, Boston, 1907.

Sumner showed that as the fish becomes enfeebled by the abnormal medium, it becomes more permeable to salts.[1] Whether the direct action of the abnormal medium, or the blocking of the gill capillaries, produce the increase in permeability, has not been experimentally tested. However, the gills themselves would not be *asphyxiated* by blocking of their capillaries, and it seems probable that the change in permeability is due to the direct action of the medium.

We may conclude therefore that the death of the fish results from the osmotic exchange. This may be sufficient to cause death while the fish still maintains its normal semi-permeability, or death may occur only after increase in permeability, due to the direct action of the medium on the osmotic membranes.

A similar increase in permeability may explain Wo. Ostwald's observations on fresh-water *Gammarus* in pure salt solutions.[2] He found that the ratio of the rapidity of death to the concentration is about constant up to a certain point, above which it is much greater. This critical concentration has nothing to do with the osmotic pressure, since it is different for different salts. Perhaps at this concentration the salt made the membranes more permeable.

Schücking[3] found that nicotine and strychnine made the skin of *Aplysia* more permeable to salts. Since cocain retarded shrinkage in hypertonic solution, he supposed that the hydrostatic pressure produced by the muscles aided shrinkage. However the hydrostatic pressure is probably very small, and the effect might have been due chiefly to an increase in permeability to salts, produced by the cocain.

3. *Secretion of Lymph and Tissue Juice.*

Höber supposes the raising of the osmotic pressure by the katabolism of the tissues, causes fluid to be drawn out of the blood-vessels, and states that the lymph in the thoracic duct has a greater osmotic pressure than the blood.

Traube states that the surface tension of transudates and exudates is always greater than that of the blood. He cites a case in which a transudate was caused to be absorbed by injecting into it a substance which decreased its surface tension.

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[1] Cf. Greene, above.

[2] *Pflüger's Arch.*, 1905, CVI., 568.

[3] *Arch. Anat. Physiol.*, Physiol. Abt., 1902, 533.

4. *Excretion.*

Milk and bile have about the same osmotic pressure as the blood, but urine is almost dry in some animals; it is usually hypertonic in man but may be hypotonic.

Traube maintains that the surface tension of the normal urine is always greater than that of the blood, and that this is the driving force in excretion.

However, Höber and others suppose that the substances to be excreted may be formed into solid bodies in the tubule cells, and thrown out into the lumen.

If lipoid-insoluble dyes are fed to frogs, granules in the cells of certain segments of the kidney tubule are stained with them. The dye is not first excreted by the glomeruli and then absorbed from the lumen by the tubule cells, for if the vena Jacobsoni, which supplies the tubules, is ligatured, no staining occurs, although the renal arteries still supply the glomeruli.

The stained granules in the tubule cells are thrown out into the lumen and pass into the bladder. These granules usually dissolve to form a slimy substance in the urine, but some of them may remain intact.

The circulation in mammalian kidneys cannot be controlled in the same way, but after intravenous injection of a certain lipoid-insoluble dye, no stain may be detected in the walls of the glomeruli, although the tubule cells are stained. The stain in the lumen does not appear above the level of the stained tubule cells. In the excretion of carmine, it may be found in granules in the tubule cells and lumen, similar to those found in frog's kidneys.

It has been supposed that urea is excreted by collecting in these granules and passing out with them, but it would be even simpler to assume that some substance is excreted into the lumen, which combines with urea and so lowers the concentration of that in solution, thus accelerating its excretion.

The chief recommendation for the granules is their valve-like action, which would account for the secretion of urine against a concentration gradient, but a simpler mechanism of such a process is shown in Hamburger's double membranes.

The blood pressure may aid in the secretion of the water of the urine, which is eliminated chiefly through the glomeruli, but its insignificance in the elimination of urea is shown by the fact that after increasing the volume (and therefore pressure) of rabbit's blood 70 per cent. by transfusion, the urea elimination was not or only very slightly increased.

VII. Cell Division.

Various hypotheses as to the cause of cell division have been advanced by the morphologists. Hertwig, supposed that when the ratio of nucleus to cytoplasm is less than normal, the cell will divide.[1] Gerassimow[2] subjected cells of *Spirogyra* to low temperatures and other abnormal conditions and obtained an increased amount of chromatin in some of them. These cells did not divide until the ratio of nucleus to cytoplasm was as great as at the time of division of a normal cell.

I found that chromatin is not necessary for cell division.[3] After extracting the chromosomes from the starfish egg, I caused it to divide. In this case the ratio of nucleus to cytoplasm was zero; however the cell did not continue to divide indefinitely.

There is no easy method of determining the ratio of nucleus to cytoplasm. Some cells contain large vacuoles whose contents are not considered as cytoplasm. Eggs contain fat drops and granules compounded of protein and lipoids. These are not considered as cytoplasm by all investigators. If the granules and oil are included as cytoplasm, the ratio of nucleus to cytoplasm is very small, and yet the egg cell does not divide unless "stimulated" by the sperm or some other means.

R. Lillie[4] observed that chemical substances, which in low concentration cause the *Arbacia* egg to divide, in high concentration cause outward diffusion of the red pigment (echinochrome) and compared this to the laking of erythrocytes.

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[1] He is not confirmed by Conklin, *Jour. Exper. Zoöl.*, 1912, XII., 1.

[2] *Bull. Soc. Imp. Nat.*, Moskau, 1904, No. 1.

[3] McClendon, *Arch. f. Entwicklungsmech.*, 1908, XXVI, 662.

[4] Biol. Bull., 1909, XVII., 188.

This is made more striking by the fact, mentioned first by Loeb, that hæmolytic agents are effective in artificial parthenogenesis. R. Lillie observed that pure solutions of sodium salts caused the egg to divide, the order of effectiveness of anions being $Cl < Br < ClO_3 < NO_3 < CNS < I$. He also found that these salts could be inhibited by others ($CaCl_2$, $MgCl_2$), as is characteristic of the antagonistic effects of salts in physiological phenomena, and the precipitation of colloids.

I found that the sea urchin's egg contains fatty substances, and relatively large amounts of lecithin probably in combination with proteids. I found that *Toxopneustes* eggs freed from the jelly-like coverings, contained about 10 per cent. lecithin (alcohol extract ppt. with acetone) and about 2 per cent. of an extract soluble in alcohol or acetone and containing rosettes of fat-like crystals. This extract blackened strongly with osmic tetroxide and effervesced on adding dry Na-carbonate in water, then emulsified, probably it contained unsaturated fatty acid.

According to a private communication by Mathews, the egg of the starfish contains lecithin and an unsaturated fatty acid, but no cholesterin. In this last characteristic it differs markedly from the erythrocyte. There is no way of determining whether these substances enter into the composition of the plasma membrane, but the facts are presented in order to indicate the possibilities.

We have seen that the exit of hæmoglobin is probably not due to increased permeability to this substance. It is possible that the same is true of echinochrome I found that the echinochrome in the egg shows a continuous spectrum, whereas that extracted in various ways shows characteristic bands. It may possibly be held by chemical combination in the egg.

However I found other evidence for increase in permeability of the sea urchin's egg coincident with beginning development:[1]

1. Fertilized eggs are caused to shrink more quickly than unfertilized eggs, with isotonic sugar solution. Presumably the fertilized eggs are more permeable to the substances exerting the internal osmotic pressure.

2. The electric conductivity of the egg increases about $\frac{1}{4}$ when

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[1] McClendon, *Amer. Jour. Physiol.*, 1910, XXVII., 240.

it is fertilized or made parthenogenetic with acetic acid, indicating increased permeability to ions.

Lyon and Shackell[1] and Harvey[2] observed that methylene blue and neutral red enter fertilized eggs more quickly than unfertilized eggs. Harvey supposed that only the free color base (undissociated) entered, since the addition of a little acid to the sea water prevented the staining of the eggs.

Mathews[3] considered the penetration of stains into the egg as a chemical process (the stain forming a salt combination with the lecithin or proteins of the egg surface).

Harvey observed, further, that NaOH penetrates fertilized more easily than unfertilized eggs, but the eggs are killed by the alkali.

The fact that the unfertilized frog's egg continues to swell *for a long time* in water (Biataszewitz) whereas the osmotic pressure of the fertilized frog's egg is *quickly* reduced to equal that of the medium (Backmann and Runnström) indicates increase in permeability to osmotic substances on fertilization. In this connection it is interesting to note that Bataillon,[4] Brachet, and myself[5] caused the unfertilized frog's egg to rotate normally and segment merely by pricking it.

It has been supposed by various observers that the "formation" of the fertilization membrane in very closely related to the segmentation of the egg. Loeb observed that the sea urchin's egg may develop without the formation of a fertilization membrane, and I have confirmed this observation, and shown that it is very probably wrong to suppose that this is a case of failure in "pushing out" of the membrane. Apparently "membrane formation" is not essential for the segmentation of the egg, although by furnishing protection it may insure the development of the embryo.

Loeb postulated that an osmotically active colloid exists in the unfertilized egg, but is so covered with lipoids that it does not absorb water until it is squeezed out or otherwise exposed at the surface of the egg, at the beginning of development (when it fills the so-called "perivitelline space"). I observed that this substance bears a positive charge (is basic) since it migrates toward the kathode when an electric current is passed through sea water containing the fertilized egg.

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[1] *Science*, 1910, XXXII., 250.

[2] *Ibid.*, p. 565.

[3] *Jour. Pharmacol. and Exp. Ther.*, 1910, II., 201.

[4] *Arch. Zool. Expér.*, 1910 (5), VI., 101.

[5] McClendon, *Amer. Jour. Physiol.*, 1912, XXIX., 298.

The unfertilized egg is imbedded in a mass of jelly which is probably mucin. This jelly bears a negative charge (is acid) since it combines with color bases.

When the positively charged colloid is exposed at the surface (on increase in permeability) and comes in contact with the negatively charged jelly, the two mutually precipitate at their surface of contact, thus forming the fertilization membrane. But if all of the jelly is washed off of the egg before the latter is caused to develop, no fertilization membrane is formed (as I have observed) because no two oppositely charged colloids are brought in contact, but the basic colloid may with difficulty be seen as a refractive layer, which has been mistaken for a poorly developed "fertilization membrane."

The observation of Lyon[1] makes it appear that catalase comes out of fertilized more quickly than unfertilized eggs, probably due to increased permeability.

Lyon observed that CO_2 came out of fertilized more quickly than unfertilized eggs, and O. Warburg, Loeb and myself[2] observed that oxygen is absorbed more rapidly by the former. We might ask: Does increased permeability allow increased oxidation, or is increased oxidation the primary cause of the increased respiration?

The permeability change is the simplest explanation, and in what other way could oxidation be increased? Loeb supposed the sperm carried an oxidase into the egg.[3] But no addition of oxidase is concerned in artificial parthenogenesis, and Loeb assumed that the oxidase (or other enzyme, kinase?) is held in the egg periphery and cannot penetrate the egg interior until the permeability is increased.

In addition to oxygen, oxidase, and escape of CO_2, hydroxyl ions are necessary for the rapid oxidation of the sea urchin egg (Loeb), and Harvey showed that the unfertilized egg is practically impermeable to OH ions of low concentration. The increased permeability allows hydroxyl ions in the sea water to penetrate the egg, as shown by Harvey, and, since the sea is always alkaline, this may explain the increased oxidation.

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[1] *Am. Jour. Physiol.*, 1909. IV., 199.

[2] McClendon and Mitchell, *Jour. Biol. Chem.*, 1912. X., 459.

[3] In this connection it is interesting to note that Masing, *Zeit. physiol. Chem.*, 1910, LXVI., 265, failed to find more iron in sperm than in sea water.

Asters always develop in the egg before segmentation. In the normal egg these have some relation to the division of the nucleus, but even if a nucleus is not present, I have observed that the cytoplasm constricts along a line on the surface farthest removed from the centers of the asters.

The constriction of the cytoplasm is probably due to a band of increased surface tension (or to decreased surface tension at the poles). This might be caused by local increase in permeability to ions, causing decreased polarization, at the equator (or increased polarization at the poles, due to increased production of the polarizing electrolyte in the asters).

The same reasons that were given for assuming that the surface of the *Amœba* is electrically polarized, hold good for the egg. The first change is probably a general increase in surface tension, indicated by rounding up of the egg. Later this may become localized from internal causes and result in cleavage.

Hyde[1] observed local changes in electric polarization of *Fundulus* eggs during cleavage, indicating that surface tension changes and cleavage are due to this cause.

It has been objected that the segmentation of the egg is not a typical case of cell division, since the egg cell is "wound up" and ready for some "stimulus" to set it going, whereas tissue cells must "grow" or "rest" after each division before dividing again.

It may be true that growth is prerequisite to division, but this cannot be formulated quantitatively. In the spore-formation of certain organisms, a cell may divide in a relatively short time into myriads of almost ultra-microscopic cells.

Hertwig may be right, in general, in assuming that the relative growth of nucleus and cytoplasm influences division, but the difficulties in proving this have been indicated, and this cannot

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[1] *Am. Jour. Physiol.*, XII., 241.

be expressed in chemical terms. It is generally supposed that nucleic acid is a more abundant constituent of the nucleus than of the cytoplasm, but much evidence has appeared for believing that it is often present in considerable quantities in the cytoplasm. Loeb supposed that the segmentation of the sea urchin egg is accompanied by an "autocatalytic" synthesis of nucleic acid, since the nuclei increased in number. But Masing[1] and more recently Shackell[2] by chemical analysis found as much nucleic acid in the unsegmented egg or 1-cell stage as in the blastula stage.

There is some indirect evidence that increase in permeability may cause an increased division rate of tissue cells. Though cell growth may influence division, it is probable that permeability influences growth.

Various "stimuli" cause increased proliferation of cells of the germinal layer of the skin. It is commonly known that mechanical stimuli increase growth of the skin.

Bernhard Fisher observed that Sudan III. or Scharlack R[3] cause increased proliferation of the epidermis. When the dye is injected under the skin of a rabbit the skin grows toward the dye.

Furst[4] found that gradual increase of temperature caused a corresponding increase in proliferation of tissue cells (due to increased chemical reaction and inflammation of the tissue). But when a certain temperature was reached a sudden jump in the increase in proliferation was observed without a corresponding increase in inflammation. This is similar to the phenomenon seen in unfertilized eggs, where a rise in temperature beyond a certain point causes segmentation.

It has also been observed that electrical stimulation may cause increased proliferation of tissue cells.

All of these changes (electrical, thermal, or mechanical stimulation, or treatment with lipoid soluble substances) cause in-

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[1] *Zeit. physiol. Chem.*, 1910, LXXVII., 161.

[2] *Science*, 1911, n. s., XXXIV., 573.

[3] Which are practically insoluble in water but soluble in fats and lipoids and, as I have observed, slightly in lipoid-protein combinations.

[4] See v. Dungern u. Werner, "Das Wesen Bösartigen Geschwülste," Leipzig, 1907, p. 65.

creased permeability and segmentation of the sea urchin's egg. Therefore, from analogy, we may conclude that increase in permeability may cause tissue cells to divide.

The "wound stimulus" to regeneration of tissue may also cause increased permeability of the cells.

In a preceding chapter it was shown that the "current of injury" produced by the negative electric potential of a wounded surface is common to animal and plant tissues. The wounded cell acts as an electric generator and a current flows through neighboring cells.

I observed that if a current is passed through living tissue, which is subsequently fixed and stained, basophile substances will be found displaced toward the anode. In sections of tissue adjacent to a wound the extent of the current is indicated by the displacement of basophile granules. The current affects first the cells in contact with the wounded cells, then extends in some directions more than others. Electric currents ("currents of growth") continue for many days after the wound has healed.

Since electric currents cause sea-urchin eggs and tissue cells to divide and proliferate, probably these bio-electric currents constitute the so-called "formative stimulus" of regeneration.

Embryonic cells, cells of germinal regions, and cancer cells are distinguished by their great power of proliferation, or rapid division. It is probable that the plasma membranes of these cells are more permeable than those of other tissue cells in the same medium or under the same conditions.

Cancers have been produced by the action of X-rays (electric pulsations) on the skin. The cells in the skin were so changed that they proliferated more rapidly. Similarly, electric changes have been observed to start the egg cell to rapid proliferation. There is probably some irreversible change in the permeability of these cells, which does not, however, make the plasma membrane incapable of subsequent reversible changes in permeability (*i. e.*, the change is unlike what occurs at death of the cell).

The suggestion that cancer cells are more permeable than tissue cells in general may possibly be of therapeutic importance. Loeb has shown that fertilized eggs are more sensitive than unfertilized eggs to various toxic substances (probably because these substances enter the fertilized eggs more easily). The same explanation may possibly be applied to the effect of sugar on certain living cells. The unfertilized eggs of the frog, *petromyzon*, sea urchin and annelid have been caused to segment, by placing them in sugar solutions. Mayerhofer and Stein[1] observed that sugar in certain concentrations increased the permeability of the gut to certain salts, and in this condition the gut was more easily injured by the diffusion of substances.

Similarly Stockard observed that sugar increased the toxicity of pure solutions of salts on the *Fundulus* egg. Morgan and Stockard[2] showed that this was not due to the inversion of sugar or to the osmotic pressure, and supposed that the sugar might combine chemically with the salt. It seems probable that the sugar increased the permeability to salt. The fact that sugar in fresh water is toxic whereas the same amount of sugar in the normal medium (sea water) is not toxic or less toxic, indicates that the salts within the *Fundulus* egg are the same as those outside (in sea water), and increase in permeability to them does not lead to diffusion while they remain in sea water, but diffusion takes place in fresh water.[3]

If it be shown that cancer cells are more permeable, substances may be found which kill cancer cells more easily than tissue cells as explained below.

Whereas a certain increase in permeability of the cell seems to cause division, a very great increase in permeability causes death (hæmolysis, cytolysis, bacteriolysis). It has been shown that certain lysins are specific for certain cells, probably because the plasma membranes of these cells differ chemically.

The fertilized egg is more easily cytolyzed than the unfertilized egg by certain substances. It therefore appears that the more permeable the cell is in the beginning, the more easily is the permeability brought to the point which causes cytolysis.

Hence it is probable that certain substances may be found by which cancer cells can be more easily cytolyzed than normal tissue cells.

[1] *Biochem. Zeit.*, 1910, XXVII., 376.

[2] BIOL. BULL., 1907, XIII., 272.

[3] In the absence of sugar I have shown that no diffusion takes place in fresh water. *Amer. Jour. Physiol.*, 1912, XXIX., 295.

It has been shown that narcosis is accompanied by decreased permeability. On the other hand, certain forms of inhibition of muscle are accompanied by an increase in permeability. May certain cells be inhibited in proliferation by an increase in permeability, too great for cell division but not great enough for cytolysis? The great oxidation rate in eggs inhibited in cleavage by very hypertonic solutions as determined by Warburg, seem to indicate this.

It has been shown that certain tissue cells inhibit the proliferation of others. In the healing of wounds, the epidermis inhibits the growth of connective tissue. If a wound remains uncovered by epidermis for a relatively long time, processes of connective tissue may grow outward, but this is prevented by the growth or transplantation of epidermis over the wound.

Perhaps the proliferation of the connective tissue is due to abnormal "stimuli" (bio-electric currents, diffusion of substances) such as cause proliferation in regenerating tissue generally. The presence of epidermis over the wound might protect the connective tissue from these "stimuli."

The foregoing facts and the speculations based on them may not be of far-reaching importance in themselves, but they suggest lines of research, which if followed, it is hoped, will add a great deal to cell physiology and pathology and be an aid to the understanding of many problems in therapeutics.

THE LARVA OF SARCOPHAGA, A PARASITE OF CISTUDO CAROLINA AND THE HISTOLOGY OF ITS RESPIRATORY APPARATUS.

WM. A. KEPNER,

University of Virginia.

The student of zoölogy is early impressed with the intensive manner in which animal life has penetrated every available space. Even so remote and strange place as the poison glands of the rattle-snake have been entered, these glands furnishing ample proteid and oxygen supply for a little nematode that makes them its habitat. In the example of this *Sarcophaga* we find a fly that has entered the nucha of the "box-turtle"—a region of the body where its larva will not be exposed to serious pressure between parts of the "turtle's" body and where it will also be free from the attacks of the appendages and mandibles of the host.

The occurrence of this parasite in *Cistudo* was first observed by Packard ('82). Packard described and figured it as in œstrid larva. Thus, so far as I have been able to determine, arose the basis for believing that a "bot-fly" infested a reptile. Aldrich ('05) in referring to Packard's paper states that perhaps it is not an œstrid. Sharpe in the Cambridge Natural History says that Œstrididæ may occur in the reptiles. Wheeler ('90) next records the occurrence of the dipteron larvæ on the nucha of *Cistudo Carolina*. He succeeded in getting the larvæ to pupate and in rearing imagines from the pupæ. These adult flies proved to belong to the genus *Sarcophaga* and not to be œstrid flies. Thus there appears to remain no evidence of a "bot-fly" infesting a reptile.

In October, 1910, a female specimen of *Cistudo Carolina* was brought into my laboratory. It was kept through the winter in a sink. January, 1911, a student called my attention to what he called a "growth" in the nucha of the right side. This, however, proved to be an insect larva. Two days later the larva escaped from the perforation made in the skin of the host.

Subsequently two other larvæ left the excavated region of the nucha. These specimens were preserved in alcohol. May 14, the fourth and most vigorous larva dropped from the host. This larva was placed upon soil in a box where it burrowed into the earth and formed an oval, dark brown pupa. This pupa has not yielded an imago, so that I have been unable to corroborate Wheeler's diagnosis as based upon the adult fly.

Except for some details which are readily overlooked in preserved specimens, such as Packard had, the larvæ I found closely resemble the figures and descriptions given by Packard. With the living material which I had at my service, I was able to see details which make these larvæ correspond more closely to the following description of larvæ of Sarcophagidæ than to that of Œstrididæ larvæ. Brauer ('83) says that the larvæ of Sarcophagidæ "are rounded, thinner anteriorly and amphipneustic. The antennæ are short, thick, cylindrical, divergent, wart-like tubercles, each with two ocellus-like chitinous rings at the tip. The mouth hooklets are distinct, strongly curved and separated from each other. The abdominal segments are distinctly differentiated by transverse swellings and are each provided with a girdle of spines. The hind stigma-plate is situated in a deep cavity, which is formed by the last segment alone. The anal swelling is two-pointed. The puparium is oval.[1]" Thus I am led to infer that I have the same kind of larva that Packard had figured and described and am able to corroborate Wheeler's statement that this is not a "bot-fly" larva but a sarcophagid larva.

Apart from this I have been interested in certain details that no one has recorded for this particular sarcophagid. Figure 1 represents the dorsal aspect of the larva magnified ten diameters. Each segment is seen to bear a band of spines. The antennæ are seen from the ventral side (Fig. 6, *ant.*) together with the strongly curved, distinct mandibles (Fig. 6). On the ventral side of the posterior segment there is a trilobed disc armed with stout spines (Fig. 3 and Fig. 5, *d*). This may function as a sucking disc. The posterior end of the last segment is divided

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[1] This translation of Brauer's description was taken from Williston's "North American Diptera," 3d ed., page 349, by Dr. J. M. Aldrich.

into a wide, dorsal lobe and a narrow, projecting, ventral lobe. Between these two lobes is a deep recess into which the anus and posterior stigmata open. The posterior stigmata are guarded by a large stigma-plate which has two lobes. Each lobe bears three spatulate chitinous bars (Fig. 4, *c.p.*) which articulate with six similar bars on the ventral lobe of the segment (Fig. 4, *c'.p'.*). The shape and relation of these dorsal and ventral chitinous bars to each other are such that I am led to believe that they function as prehensile structures; the lower lobe of the segment pressing its bars against the bars of the stigmatic plate can lay hold of the wall of the excavated region in the skin of the host and thus anchor the larva. The most striking feature to which attention has not been called is the presence of two anterior stigmata (Fig. 1, *st.*). These stigmata are fan-shaped structures which bear seventeen or eighteen papillæ along their terminal edge (Fig. 2, *st.*). In a specimen cleared with xylol each of these stigmata can be seen to lead directly into a large lateral trachea. Thus they are provided with an air-breathing apparatus though they live in a thick fluid of suppurated matter which makes liable the clogging of one or more of these tracheal openings or may necessitate the temporary closing of one of them. In this connection it is interesting to find a transverse tracheal commissure posterior to the anterior stigmata and another transverse tracheal commissure anterior to the posterior stigmata. These commissures enable both tracheal trunks to get air though for any reason some of the stigmata may be closed. Thus the chief tracheal system consists of a pair of anterior and a pair of posterior stigmata and two lateral tracheal trunks which are connected by means of an anterior and a posterior tracheal commissure.

Nothing unusual has been noted concerning the histology of the tracheal trunks and posterior stigmata. The histology of the anterior stigmata has, however, attracted my attention. These fan-shaped structures are for the most part proliferated masses of cuticle. The anterior half of the stigma projects beyond the contour of the body as a stigmatic process. The posterior half lies beneath the surface of the body and is covered by an epithelium which represents the hypodermis modified as tracheal epithelium (Fig. 8, *te.*). From the posterior margin of the stigmatic process there is a cuticular and hypodermal invagination which extends to near the base of the stigma as a retaining thread (Fig. 8, *inv.*). This retaining thread of cuticle and hypodermal epithelium is seen in transverse section at *inv.* in Fig. 9. The entire stigma represents a modified region of hypodermis and cuticle. On the mesial side of the stigma near the base of its anterior third the hypodermis becomes very pronounced, the cells becoming very large and columnar. These cells, so far as their form is concerned, are the most conspicuous tracheal cells (Fig. 7, *te.*). From them slender processes go into the cuticular mass of the stigmatic process. These processes and the position of these cells suggest that they not only help to elaborate the cuticular substances of the stigmatic process but that, also, they may be able to move the stigmatic process. Within the mesial wall of the stigmatic process no cytoplasm extends except that of these cellular processes; within the lateral wall of the stigmatic process scattered hypodermal cells are found. There is thus an indifferent cellular supply to the tracheal process of the stigma. Indeed the entire stigma is for the most part a cuticular structure. The cuticle of the general surface of the body is distinctly two-layered. The outer layer is the deeper and in hæmotoxylin stains the more deeply. The inner layer is clearly a softer substance and does not stain deeply. These two strata are involved in the formation of the anterior stigma. The inner layer, except for becoming more abundant in the stigma, is not modified. Figure 7 at *c* and Fig. 8 show this layer of the cuticle passing over into that of the stigma. The outer layer of cuticle, however, is thinner over the stigmatic process than over the general surface of the body. When it reaches the tips of the papillæ it is invaginated and passes as a series of converging tubules to the bases of the papillæ where the tubules unite to form a large tube whose lining is confluent with the lining of the tracheal trunk. The cuticular lining of the tracheal trunk also presents a deeply staining layer and a layer that does not readily stain (Fig. 11, *tl.*), thus resembling the cuticle, of which I believe it represents a modified region. The inner denser layer of this tracheal lining gives rise to spiral tænidia as shown in Fig. 11 at *t*. When this denser layer passes into that of the stigma very minute slender processes arise from it into the lumen: these processes branch and rebranch to form a reticulated layer which takes the place of the tænidia of the trachea (Fig. 10, *r.*). This reticulated layer is increased until the entire lumen is filled with a reticulated mass or plug (Figs. 8, 7, and 9, *rp.*). At the base of each papilla the reticulated plug branches and continues to near the tip of the papilla where there is a small chamber into which the branch of the reticulated plug sends its terminal filaments (text-figure 1). Thus we find the cuticle, tracheal lining and the cuticular mass of the stigma to be two-layered. In all three places the non-staining layer is little modified; but in the tracheal lining the deeply staining layer is modified to form the tænidia, and in the tracheal process it becomes a reticulated plug.

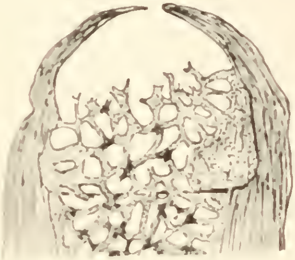

FIG. 1. Longitudinal section of a papilla of the anterior stigma, showing the terminal chamber into which filaments of the reticulated plug project. × 1,500.

The larvæ of blow-fly and house fly have likewise prothoracic stigmatic processes with finger-like papillæ. These in turn, according to de Meijere ('02), have reticulated plugs which he calls "felt-chambers" (Feltkammern). What does such histological structure mean? We see the cuticular hairs guarding the stigmata of ants or other insects and we interpret them as being devices to protect the trachea from foreign bodies. But here we have in place of protecting hairs an extensive, finely reticulated plug which resembles the cotton plug of a bacterial culture tube as though it were constructed for the purpose of protecting the trachea from microscopically minute bodies. The larva feeds upon the suppurated fluid found within the excavated region of the nucha of the host, hence while the larva is feeding these bacteria can hardly be of service, for the anterior end of its body is bathed in the suppurated mass. However, when about to pupate the larva reverses its position with reference to the suppurated mass, and lies with its anterior end directed towards or through the opening in the skin of the turtle. The larva is then in a position to breathe air through the anterior stigmata. At the same time the larva during the three or four days spent in emerging from the host, frequently retreats into the excavated cavity when disturbed, thus its anterior end may repeatedly become contaminated with the bacteria of the suppurated mass. I think, therefore, that the anterior stigmata are chiefly functional during the two or three days spent by the larva in passing from the turtle to the ground and that the reticulated plug is a bacterial screen protecting the trachea from infection threatened by the repeated retreat of the larva into the excavated cavity when it lies with its posterior end at or within the suppurated mass. If this conjecture concerning the time and character of the functioning of the anterior stigmata is not warranted, I believe that I am justified in agreeing with Hewitt ('08), that the anterior stigmata of this character are functional at some stage in the life of the larva.

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Explanation of Plate I.

Fig. 1. Dorsal aspect of larva. *st.*, stigma. × 10.

Fig. 2. Lateral aspect of anterior end of larva. *mo.*, mouth; *m.*, mandible; *st.*, stigma. × 100.

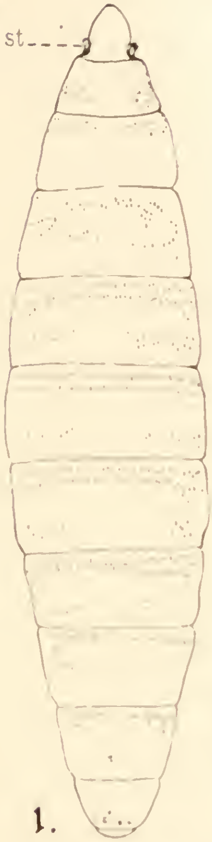
Fig. 3. Ventral aspect of posterior segment. *d.*, tri-lobed disc with stout spines. × 25.

Fig. 4. Ventral aspect of posterior segment. The ventral lobe is laid back so as to expose its six chitinous bars *c′p′*., and the two-lobed stigma-plate with its six chitinous bars *cp*. × 25.

Fig. 5. Lateral spect of posterior end of larva. *d.*, tri-lobed disc; *cp.*, chitonous bar of stigma-plate. × 10.

Fig. 6. Ventral aspect of anterior end of larva. *m.*, mandible; *mo.*, mouth; *st.*, stigma; *ant.*, antenna. × 25.

Fig. 7. Transverse section through base of tracheal process at level indicated by arrow 7 on Fig. 8. *c.*, cuticle; *rp.*, reticulated plug; *h.*, hypodermis; *te.*, tracheal epithelium. × 250.



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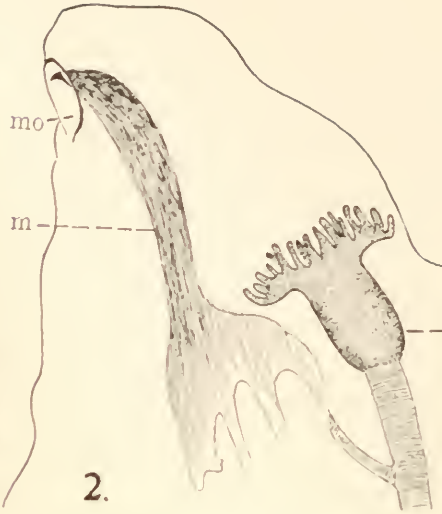

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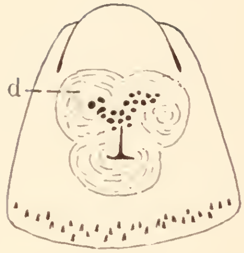

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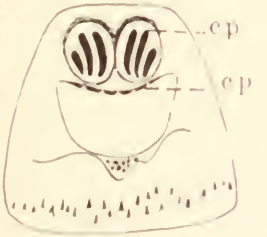

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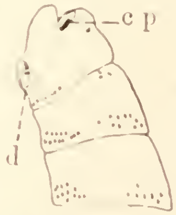

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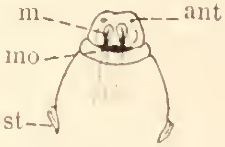

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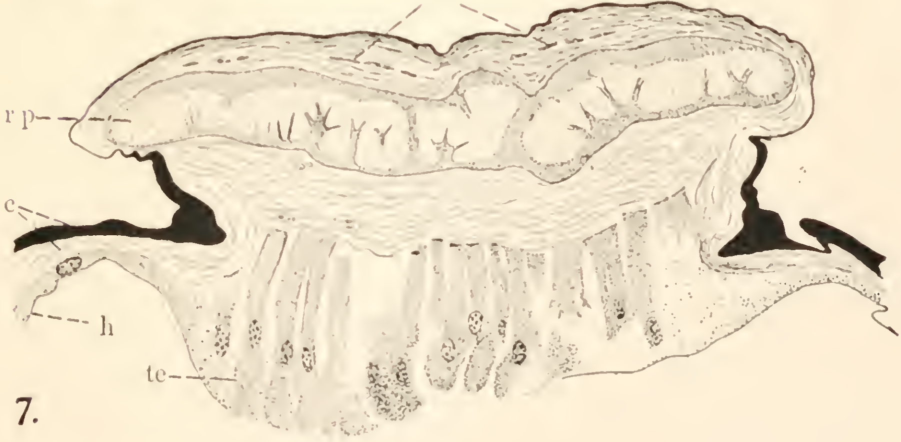

7.

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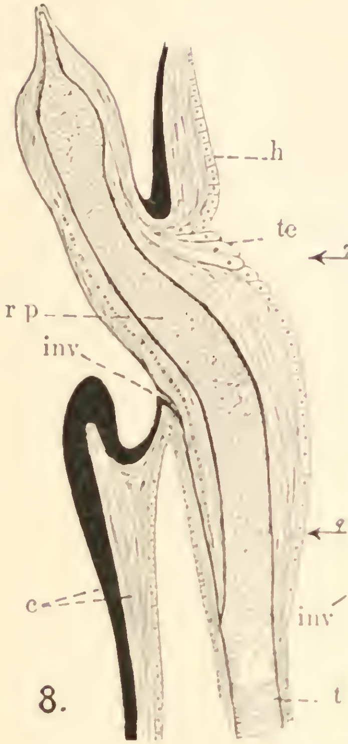
EXPLANATION OF PLATE II.

FIG. 8. Reconstructed drawing of anterior stigma. *h.*, hypodermis; *te.*, tracheal epithelium; *t.*, tænidia; *c.*, cuticle; *rp.*, reticulated plug; *inv.*, invagination of cuticle. × 200.

FIG. 9. Transverse section of trachea through level indicated by arrow 9. It shows the secondary invagination with its cuticular core *inv.*, *rp.*, reticulated plug; *te.*, tracheal epithelium. × 500.

FIG. 10. Part of trachea in the transitional zone between the reticulated plug and the tænidia of the trachea. *r.*, reticulated chitin arising from the denser layer of chitin; *te.*, traceal epithelium. × 1,500.

FIG. 11. Part of wall of trachea. *t.*, tænidia; *te.*, tracheal epithelium; *tl.*, tracheal lining. × 1,500.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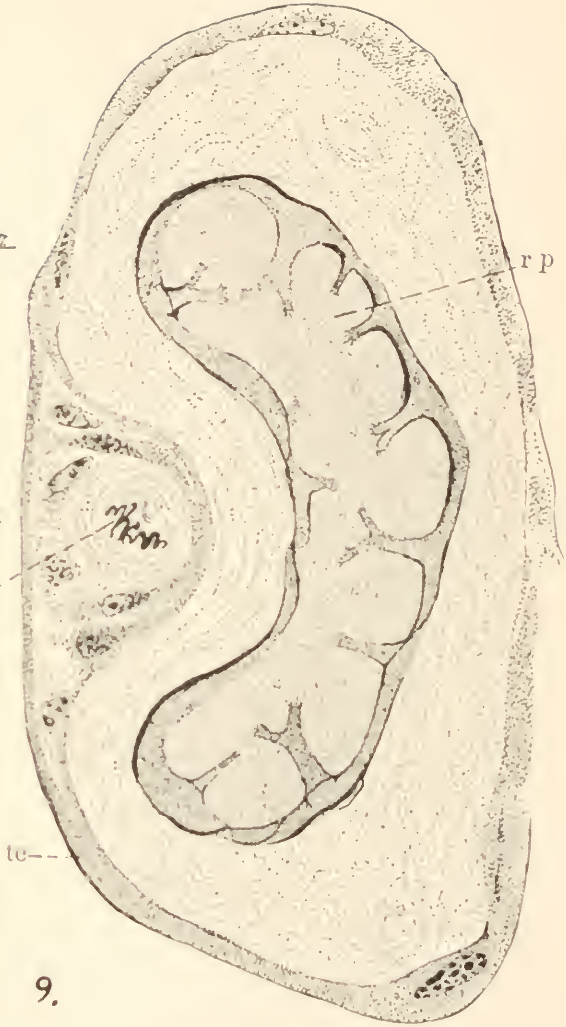

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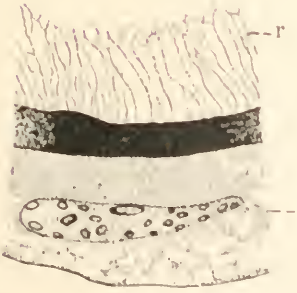

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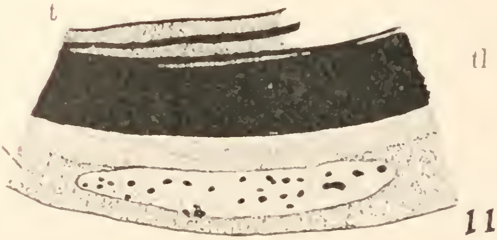

11.

WM. A. KEPNER

EARLY DEVELOPMENT OF GRAFFILLA GEMELLIPARA —A SUPPOSED CASE OF POLYEMBRYONY.[1]

J. THOMAS PATTERSON.

I. Introduction.

In the Brooks' Memorial Volume of the *Journal of Experimental Zoölogy*, Vol. 9, 1910, Professor Edwin Linton reports the discovery of a very interesting viviparous rhabdocœle commensal with the common ribbed mussel, *Modiolus plicatulus*, found along the Atlantic coast. Dr. Linton refers this worm to the genus *Graffilla*, and on account of its peculiar method of producing embryos in pairs, designates it by the name *Graffilla gemellipara*. So far as we know the only other statement in the literature that could be interpreted as referring to this interesting turbellarian is found in a short paper by Nicoll, '06, entitled "Notes on Trematode Parasites of the Cockle (*Cardium edule*) and Mussel (*Mytilus edulis*)."

Nicoll figures (in his Fig. 7) what he calls a trematode sporocyst from the liver of the cockle, but it is quite clear from Linton's work that he is in error in calling this specimen a sporocyst. What he in all probability had was a specimen of a species of turbellarian closely related to if not identical with *G. gemellipara*. This is evident from the fact that his figure shows the presence of paired embryos, as well as a pharynx, which alone would exclude the case from the category of sporocysts.

Linton's paper gives an account of the more general features of the worm, but leaves several important questions unanswered, among which may be mentioned the following: (1) How is the yolk deposited in the ova? (2) How do the sperms reach the "sperm-sac"? (3) Is the species protandrous? (4) Where are the eggs fertilized? (5) Finally, and most important of all, How do the two embryos in each capsule arise?

In regard to this last point, Linton suggests that we may have a case of polyembryony. It was this suggestion that induced me to undertake a study of certain phases of the development of *Graffilla;* and this not only because of my interest in the general subject of polyembryony, but also for the reason that an opportunity seemed to be offered to work out the details of this peculiar phenomenon. Furthermore, if a true gemelliparous development really did exist in so simple a fashion in a relatively low organism like *Graffilla*, it might be possible to modify experimentally the process and thus to be able to get at some of the factors underlying it.

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[1] From the Marine Biological Laboratory, and the Zoölogical Laboratory of the University of Texas. Contribution No. 109.

While the results obtained from these studies have proved disappointing, at least so far as the main object for which the investigation was undertaken, yet they are of a character such as to warrant record, especially as they answer satisfactorily some of the questions raised above. Furthermore, we have as yet only a very few papers dealing with the development of rhabdocœles, and consequently there is need of contributions along this line.

Methods.—Various methods for preserving the material have been used, but the most successful fixing fluid has been found to be Benda's modification of Flemming's strong solution. Specimens fixed for two hours in this fluid give beautiful results for cytological study, especially when followed by iron-hæmatoxylin stains. Bouin's fluid also gave good preparations, but is much less certain in its results. In making whole mounts the specimens are placed under slight pressure and killed over a gentle flame, and then fixed in a corrosive-sublimate solution. If followed by borax carmine such material gives very clear figures of many structures. However, I find the same "indefiniteness" about the reproductive organs as noted by Linton, especially in regard to the ducts, so that one can not rely upon mounts for one's interpretation of the conditions of these structures.

Notes on the Habits.—Linton states that *G. gemellipara* lives on the gills of *Modiolus*, but there is some evidence that they inhabit the kidney. This is brought out in the following experiment. Two dozen specimens of *Modiolus* from a lot yielding no *Graffillæ* from the gills were opened, care being taken not to injure any of the tissues, and thoroughly washed out in water. No parasite was found. The kidneys of these same individuals were teased out and the specimens again washed in water, with the result that thirty-eight *Graffillæ* were secured. Undoubtedly many individual parasites escape from the kidney of the host and are later found in the mantle cavity and on the gills, and this would account for their discovery there by Linton. Furthermore the method ordinarily employed in opening the molluscs would necessarily result in injuring the kidney, and thus permit the escape of the parasite from that organ. The experiment mentioned above would seem to indicate clearly that *G. gemellipara* is a true endoparasite, but the experiment was performed at the close of the season and the opportunity was not offered to settle the question conclusively, as that could only be done by making careful dissections of the individual molluscs. We should expect to find this species of parasite in the kidney or liver of the host since all of the other species of the genus *Graffilla* are found in the same organs of the various molluscs.

The best season of the year in which to secure *G. gemellipara* at Woods Hole is during August, from the 10th to the 20th of the month. Specimens may be obtained prior to this, but they are usually immobile individuals which contain numerous young that have liberated themselves from their capsules and are swimming about in the mesenchyme. Such material is valuable for obtaining very young animals. On July 5, 1911, several of these exhausted mothers were placed separately in hanging drops of the fluid taken from the mantle cavity of *Modiolus*. The cover slip from which the drop was suspended was placed above the cavity of a hollow ground slide and sealed with vaseline. In this way the specimens could easily be studied under the microscope. On the following day it was noted that most of the young had ruptured the wall of the mother and were swimming about in the drop. In one case the escape of the young was actually observed. Young animals secured by this method can be kept alive without much trouble for about two days, and undoubtedly would live longer if proper care were taken. However, it was found unnecessary to obtain material for study in this way after forty-eight hours, for the washings of *Modiolus* yield many young specimens that correspond in size to these two-day old worms.

An interesting periodicity in the reproduction of *G. gemellipara* occurs at Woods Hole. From the 20th to the 25th of June (1911), shortly after the writer arrived there, specimens were secured in considerable numbers, but from this date until about the 10th of August it was extremely difficult to obtain material, although molluscs from many different regions were examined. From an entire bucketful of the *Modiolus* not more than a dozen would be secured, and these were either very young, sexually immature animals, or very large individuals which were about on the point of undergoing degeneration and freeing their young. About the middle of August, both in 1910 and 1911, *Graffillæ* were secured without difficulty, but from the 25th of the month until the 12th of September, when I left Woods Hole, they were extremely scarce. From this it would seem that there are two summer periods of rapid multiplication, one in June and the other in August; and possibly a third period occurs in October. Linton reports that Coe found *Graffilla* in abundance at New Haven during the month of October.

At no time does one find *G. gemellipara* in such numbers as reported by some of the writers on the other species of the genus. Jameson, '97, states that from four to several dozen individuals of *G. buccinicola*, which is parasitic in the kidneys of *Buccinum undatum* and *Fusus antiquus*, are found in every specimen of the two molluscs.

II. Structure of the Reproductive Organs.

The reproductive organs of this *Graffilla* are difficult to make out, both on account of the viviparous method of reproduction as well as on account of the variability in the development of the different parts. *G. gemellipara*, like certain other members of the genus, exhibits successive hermaphroditism, but the case is not so extreme as that described for *G. buccinicola* by Jameson, '97. The male organs develop first and upon reaching their maturity at a comparatively early period in the post-natal life, in part atrophy, and are then followed by the development of the female organs.

The male organs consist of the following parts: (1) a pair of testes which lie just posterior to the pharynx, one on each side of the median line somewhat below the central axis of the animal (Fig. 1); (2) two very delicate, short sperm ducts which place the gonads in communication with the seminal vesicle; (3) a seminal vesicle, which is a rather large pear-shaped sac situated just below the genital pore; and finally, a plug-like penis arising from the pointed, ventrally directed end of the seminal vesicle. In one of the clearest specimens secured each sperm duct is seen to arise from the posterior median corner of the testis and to pass inward to the anterior face of the seminal vesicle, meeting the latter at about the dividing line between its upper, bulbous portion and the smaller lower part. The penis when contracted is extremely difficult to make out, and since in mounted preparations this condition is almost invariably met with, not many of the details of the organ were studied. The penis when extended of course protrudes into the common atrium, which in turn communicates with the exterior by means of the small genital pore. The pore lies in the median ventral line at a point situated about one third the distance from the anterior end of the body.

In large individuals the testes are seldom found, and when present are mere degenerating fragments. The seminal vesicle, however, persists at least until a late period of the post-natal life, but in many animals becomes reduced in size. The penis also degenerates sooner or later. During this period of degeneration of the male organs the female reproductive structures gradually make their appearance. One occasionally meets with specimens in which the transition from the "male" to the "female" state is seen, and from such individuals most of the important points concerning the female organs can be made out.

In the typical "female" condition the seminal vesicle is always present, though as stated above it may become greatly reduced in size, and the atrium with its genital pore still persists. Just back of the seminal vesicle and dorsally the atrium gives rise to a small diverticulum, which both from its position and character suggests its homology with the receptaculum seminis of the other members of this genus, although in the two clearest cases coming under the observations of the writer this vesicle contained no spermatozoa (Fig. 2). If this interpretation is correct then the receptaculum seminis is in this species clearly a degenerate structure.

Posteriorly the atrium is directly continuous with an enlarged, rather thick-walled uterus, which in turn gives rise to a duct-like structure that extends backwards and upwards (Fig. 5, *u*). At the point where these two parts join, the uterus receives the small ducts of the many unicellular shell-glands (Fig. 1, *s*).

Towards its distal end the uterus bifurcates, sending a branch to each of the bilaterally arranged ovaries (Fig. 3). The bifurcated part of the uterus serves as a receptacle for spermatozoa —a condition that is not entirely unique for this species—and also performs the function of insemination. On account of the backward and upward course taken by the uterus, the two distal parts come to lie just below the ventral surface of the intestine, at a place slightly posterior to the middle point of the body (Fig. 5).

The development of the uterus has not been studied and I can not therefore state with certainty the exact nature of this organ. Slightly posterior to the point where the proximal and distal parts join the duct is frequently very indefinite and difficult to trace. This, together with the fact that small yolk cells are frequently found within its cavity (Figs. 4, 5) has led the writer to believe that the distal part of the uterus is the product of fusion between the ducts coming from the reproductive glands and therefore should probably be called the oviduct.

The female reproductive glands consist of a paired "germarium" and a paired "vitellarium," the two glands on each side being so closely associated that the compound structure might properly be termed a "germ-vitellarium." The ovarian portion occupies the anterior part of the body, while the yolk glands occupy the posterior half mainly.

The clearest idea of the relation of these various parts to each other and to the reproductive ducts can best be gained in a study of horizontal sections which pass just below the ventral side of the intestine. In such sections the ovary on each side is seen to begin slightly anterior to the seminal vesicle, and to increase gradually in diameter in passing backwards until it reaches the region occupied by the distal end of the uterus. Here it spreads as a fan-like structure, with the inner margins of the ova converging to meet the tip of the uterus (Fig. 4). In composition the ovary is made up of flattened cells, and one might compare it to a rouleau of coins of gradually increasing size, the smallest being located at the anterior end. The larger cells of the ovary are produced by the absorption of nutritive materials from the vitelline cells, in a manner that will be described in the next section.

The vitellarium is an extensive organ, and in the posterior half of the body almost completely envelops the intestine (Fig. 6). In the early stages of its formation the cells are very similar to those of the ovary, and even in the definitive condition their nuclei have the characteristics of ovarian nuclei. The ovarian and vitelline cells are in very close association at the middle region of the body, and for some little distance anterior to this the ovary is overlaid by the yolk cells.

III. Early Development.

1. *Nutrition of the Ova and the Formation of the Egg-capsule.* — In order to be able to understand clearly the manner in which the ova are nourished and the egg-capsule is formed it is necessary to call attention to the characteristic condition in *Graffilla* of the duplexity of embryos in each capsule. In all of the older stages the two embryos are surrounded by a very thin transparent membrane or shell inside of which the two ciliated individuals may move about each other with considerable ease. In late cleavage, or indeed in any stage of segmentation, this thin shell in the strict sense of the word does not exist, though the outermost portion of the yolk is of a consistency such that it serves the purpose of a shell, and out of this surface layer the true shell doubtless differentiates. During the cleavage stages it is seen that a considerable mass of yolk surrounds the two embryos (Fig. 10). The two embryos may be either close together, with only a very thin intervening layer of yolk, or widely separated and situated at the extreme opposite ends of the capsule (Figs. 2, 3). In either event the most pertinent question that one can raise is how the two embryos have come to exist within the same yolk mass.

As we have pointed out in the preceding section, the ovaries are at their posterior ends somewhat closely approximated on the ventral side of the intestine, and are intimately associated with the yolk glands, being surrounded on the dorsal and posterior aspects by them. In a longitudinal section of almost any individual in the egg-producing stage one can observe that the ova are at their upper margins absorbing yolk from these glands, and while the nutritive process may involve the ova of one half of the ovary, yet it is much more conspicuous in the posterior third of that organ (Fig. 9). At the extreme end of the ovary the absorption goes on with great rapidity, the ova soon becoming gorged with nutritive material. In consequence of this rapid growth certain retrogressive changes involving the cell membranes separating contiguous ova frequently make their appearance. As a result two or even more nuclei may come to lie within a common yolk mass, which occupies the extreme tip of the ovary (Figs. 9, 10). In other words, a syncytium is formed here. In the vast majority of cases only two ova are involved so that the usual picture displayed in this region represents a binucleated yolk mass (Fig. 15).

It should be noted here that in this peculiar method of nutrition we have a mechanism alone adequate to account fully for the reason why two embryos are habitually borne within a single capsule. Just why two should appear is difficult to answer. As a matter of fact, however, two are not always present, for as Linton has pointed out capsules are sometimes seen with three embryos, and a few cases were noted by him in which only one embryo is surrounded by the envelope. Furthermore, in the figure of Nicoll referred to above, two capsules containing three embryos each are clearly shown. In my own material several cases of "triplets," including one with undivided eggs, have been observed, as well as several with one embryo each. While in the light of these facts the twin condition in *Graffilla* loses much of its apparent significance, yet its appearance in the great majority of cases made it necessary to undertake a careful study of the histogenesis of the ovary in order to see if any mechanism, other than that of the breaking down of intervening membranes, could be discovered that would explain a potency to gemelliparous reproduction on the part of that organ. At first it seemed probable that a binucleated ovum was produced somewhere in the oögonial history. A diligent search in the ovary fails to reveal any binucleated ova, except of course at the extreme tip, nor has the slightest evidence been secured of nuclear divisions either mitotic or amitotic throughout the entire length of a fully matured ovary. We are therefore forced to the conclusion that what we have described in connection with the absorption of yolk furnishes the key to the twin condition in *Graffilla*. It can not be argued that the breaking down of the membranes is only apparent and therefore an artifact produced by reagents, for it has been observed in preparations made from material preserved in a dozen different fixing fluids, and followed by as many different stains. However, not in all cases do the two contiguous ova lose their intervening membranes, but some become completely surrounded by vitelline cells, which through a process of disintegration form the yolk mass of the definitive capsule (Figs. 7, 16). In such cases the two ova do not lose their "individuality," and a subsequent reorganization of new membranes about the two nuclei will not take place. Considerable evidence has been secured which indicates that these two methods of capsule formation are but the extremes of one and the same process.

Throughout the entire history of yolk absorption many interesting changes, involving both the nucleus and cytoplasm, are seen, but we can not deal with all of them here Our attention must therefore be directed to those that seems to us to be most important.

In Fig. 14 is represented a pair of nuclei lying within a single membrane. The lower of these is immediately surrounded by a layer of finely granular protoplasm, about which one can trace another very delicate, but nevertheless distinct, membrane. This condition has been observed in a number of ova, and may begin before the binucleated stage is reached, that is, in ova situated from two to six cells from the tip of the ovary. I have not been able to demonstrate the universality of this membrane, and I am therefore inclined to regard it as the intra-cellular or intra-vitelline membrane that is sometimes laid down about the ovarian nucleus. It may be that in *Graffilla* it marks the beginning of the segregation of the protoplasm from the yolk, and is therefore the first step in the reorganization of a cell about each of the nuclei in the capsule.

In Fig. 9 is seen the last trace of the intracellular membrane in a binucleated mass that is about ready to be freed from the ovary. It is possible of course that the faint line about the large nucleus is not an intra-cellular membrane, but only the original cell-wall which has become much attenuated through the absorption of yolk by the ovum. This figure is of further interest in that it demonstrates with remarkable clearness the manner in which the yolk is absorbed by the ova. At the extreme end of the ovary the process is at its height, and one can actually observe the configuration of the streams of food material extending from the vitelline cells to the larger nucleus. This is particularly true in the pseudopodial-like structure in the upper median portion of the figure. On the extreme right, near the section of the tip of the second nucleus, the yolk cells are directly open to the ova. It is not quite clear as to what extent the yolk cells participate in the formation of the mass of yolk surrounding the eggs, aside from merely giving up their nutritive materials; but that they do assist in this formation is abundantly proved in those capsules the yolk contents of which show many degenerating nuclei of vitelline cells. In some cases these fading nuclei form a complete row just below the surface of the capsule.

Some half dozen cases have been found in which the ovum apparently does not become surrounded by any considerable amount of yolk, but after absorbing a small amount of food material is set free from the ovary. These single naked eggs float about in the parenchyma and probably never succeed in producing embryos (Fig. 13).

Some time prior to the liberation of the ova from the ovary and the yolk-gland, the ovarian nuclei undergo marked changes. During all of the preceding oögonial history the nucleus possesses that characteristic coarse network of chromatin extending throughout the nucleoplasm, and a very large, deeply staining nucleolus (Fig. 9); but during the last stages of yolk absorption the chromatin network becomes more or less indistinct (Fig. 7), finally disappearing altogether, and in its stead a finely granular condition of the chromatin appears. At the same time the nucleolus stains less intensely and soon becomes very irregular in outline (Fig. 10).

It is necessary to mention only briefly the manner in which the "ovulation" takes place. By the time the absorption of yolk has reached the point seen in the case of the ova on the right of Fig. 9 the formative capsule may be said to be practically independent of any ovarian connections, and it only remains for the capsule to be freed from the vitellarium. However, its attachment with the yolk glands persists for some time after this, even indeed until the two eggs reorganized, if reorganization is necessary. In Fig. 10 is a capsule just about ready to be set free into the parenchyma; most of the yolk cells have yielded up their food contents to the capsule, and the region immediately surrounding its upper margin shows only delicate strands connecting it with a few of the remaining nurse cells. Shortly following this period the strands are severed and the capsule rounds up, and as the whole structure is pushed about in the parenchyma by the movements of the mother worm the eggs undergo development.

Up to the present we have been using the term "capsule" to mean the whole yolk mass surrounding the two eggs; and we must now consider briefly the formation of the thin capsule or shell, by which we mean the membrane containing the two ciliated embryos of the later stages. Since the eggs with their follicular layer of yolk do not enter the uterus, it is not probable that any of the secretions from the unicellular shell-glands reach the eggs and thus take part in the formation of the shell, as occurs in the case of oviparous forms. I have not followed all of the steps in the formation of the shell, but it has been observed that as development proceeds the outermost layer of the yolk, which at first is very plastic and yields readily to any obstruction in the parenchyma, gradually becomes more resistant, finally taking on the thin elastic character met with in all of the advanced stages. It is probable that the shell is in part the product of the parenchyma.

It remains to say a word about the "reorganization" of cells in those cases in which the membrane in part or completely disappears from the two ova. Even in the extreme cases it is doubtful whether the cytoplasmic part of the cell becomes indiscriminately associated with the yolk portion of the capsule.

This part of the study has furnished many difficulties, because of the fact that the capsule at this particular stage is very plastic and hard to fix properly. Only a few cases of good fixation have been secured: and in one of the clearest of these the nuclei are seen to be surrounded by a finely granular protoplasm, about which a membrane must later be secreted.

2. *The Aborting Spindle.*—The study of maturation and fertilization was made difficult by the presence of a spindle which appeared in the egg some time before the egg capsule was set free into the parenchyma. On account of its large size the spindle was at first taken to be that of the first cleavage, but inasmuch as the first division of the fertilized egg results in cutting off a small micromere, it soon became evident that this interpretation was incorrect. Furthermore, in the eggs in which the large spindle appeared the most diligent search failed to reveal any polar bodies. When this fact once became fully established it was evident that we had in *Graffilla* a display of that remarkable phenomenon of a "disappearing" or "aborting" spindle, first discovered by Selenka, '81, and to our knowledge of which Wheeler, Gardiner, and others have contributed.

Selenka's discovery was made in connection with his work on the polyclad *Thysanozoön Diesingii.* He describes the aborting spindle as appearing in the uterine eggs. After the egg has reached its full growth, the germinal vesicle begins to make preparations to divide in the typical manner; the chromatin forms a spireme, the achromatic spindle with its two centrosomes appears, and the chromosomes pass into the equatorial-plate position. At this point the process stops, and the nucleus returns to a resting condition. Subsequently the egg throws off two polar bodies, is fertilized, and develops in the normal manner. Inasmuch as the yolk granules are evenly distributed throughout the egg at the beginning of this peculiar phenomenon and are collected about the astral centers at its close, Selenka supposes that the function of the aborting spindle is to mass the granules at the center of the egg. But this interpretation fails to explain the appearance of the spindle in those eggs in which a collecting of the granules about the astral centers does not take place, as both Lang and Wheeler have observed.

Lang, '84, next noted the aborting spindle in several polyclad eggs, and figures it in the uterine egg of *Thysanozoön Brocchii.*

Wheeler, '94, describes briefly the appearance of the uterine spindle in the eggs of *Planocera inquilina*, a polyclad inhabiting the branchial chamber of *Sycotypus canaliculatus*, but does not attempt to work out the details of the process. He also noted the spindle in the eggs of the acœlan *Polychœrus caudatus.*

Gardiner, '95 and '98, working on the latter species came to the conclusion that the aborting spindle is abnormal, representing the first cleavage spindle of eggs retained too long in the uterus of an animal kept under abnormal conditions. His point does not seem to be well taken, as Surface, '07, has shown in his work on *Planocera.*

The last reference to the aborting spindle that we may note is that of L. von Graff, '82, in his monograph on the Rhabdocœlida. Von Graff, although making no reference to the spindle in the text, clearly figures one in the uterine eggs of *Aphanostoma diversicolor* and *Cyptomorpha saliens.*

In our species, *G. gemellipara*, the aborting spindle appears in the eggs some time before the freeing of the egg-capsule from the vitellarium. The spindle is really anticipated long before all of the yolk is laid down about the two eggs, as can be seen in Fig. 19. In many respects the spindle is truly remarkable, not only on account of its great size, but also for the reason that frequently the chromosomes do not appear upon it. One of the clearest cases that has come under my observation is shown in Fig. 17. This is an especially well preserved egg, yet one can not detect the slightest trace of chromosomes in the cell. However, it is probable that the chromatin is represented by some of the central spindle fibers, which are quite thick but do not take the stain well. This is most certainly the case in some eggs in which very delicate chromatin threads among the spindle fibers can with difficulty be made out.

Sometimes the chromatin is in the form of chromosomes, which however are not located on the spindle. In Fig. 18 is shown such a case. Here the large conspicuous spindle is itself free from chromatin, but among the astral rays of one end are four chromosomes, which are of interest not only because of their peculiar position, but also because they are apparently bivalent. They are not tetrads in shape, as in the characteristic condition of the first maturation, yet that they are the egg chromosomes and not those of the sperm is evidenced by the fact that the sperm is located in another part of the ovum.

The peculiar behavior of this karyokinetic figure is not confined to the chromatin; the centrosomes frequently present unique conditions. It is not uncommon to find the centrosome at one or both ends of the spindle undergoing division, but this would not be striking—since in many germ cells, both male and female, a precocious division occurs—were it not for the fact that at one end the axis of the two centrosomes is at right angles to that of the spindle, while at the other end it is simply a continuation of the spindle axis. The precocious division of the centrosome frequently results in the formation of a double aster.

I have not been able to follow with certainty all of the subsequent steps in the history of this spindle, but the end result in all cases would seem to be a return to a sort of resting stage on the part of the nucleus. It differs from the corresponding stage of *Thysanozoön Diesingii*, in that the nucleus instead of being a large vesicle, appears in the form of four vesicles, one for each chromosome (Fig. 19). These may be more or less grouped together or widely separated, but they later come together and fuse, producing a lobulated nucleus which retains this condition until the onset of maturation (Fig. 21). It will be seen from this rather brief account that the only function which one might assign to the aborting spindle in *G. gemellipara* is that of scattering the chromosomes in the form of vesicles; but since these are later collected together into a single vesicle before maturation, it is difficult to attach any real significance to this whole peculiar phenomenon. Inasmuch as several odd conditions have been observed, both in the centrosomes and the chromosomes, it is not at all improbable that the aborting spindle is an abnormal display. But it can not be the result of placing the animals under unfavorable conditions because the spindles are found in worms killed immediately upon their removal from the mollusc.

It should be pointed out here that *Graffilla* is not a favorable form in which to work out the history and significance of the aborting spindle, for owing to the viviparous mode of reproduction prevailing in this species it is quite impossible to secure a complete series of stages showing the different steps. One can not be at all certain that it occurs in every egg, though the frequency at which it is met would indicate that it did. Nevertheless, it would seem that some rather important function should be assigned to the aborting spindle; for its appearance in some dozen different species of flat worms must exclude it from the category of abnormal behavior. It is therefore hoped that an opportunity may be offered to work out its history in detail in a favorable form, such as one of the oviparous species from which a series of stages can be secured from the uterus.

3. *Insemination.*—By insemination is usually meant the act of introducing the spermatozoa into the egg. In *Graffilla* the process occurs during the last stages of yolk absorption while the formative capsule is still attached to the ovary, and consists in the introduction of spermatozoa into this capsule. The inseminating organ is the modified, or bifurcated part of the uterus. In Fig. 6 is shown a beautiful case. The section passes through the distal end of the uterus, and the left-hand lobe of that organ, filled with spermatozoa, is in direct contact with the binucleated capsule. Any number of similar figures can be demonstrated in the preparations, so that no doubt can exist regarding the interpretation which we have placed upon such pictures. It would seem that the uterus took an active part in the process of insemination. Linton reports an observation which points to the same conclusion.

This method of insemination must necessarily permit a number of spermatozoa to get into the capsule, but owing to their small size they are soon lost among the yolk granules, so that an enumeration of them is impossible. So far as one can tell the sperms do not at first invade the immediate neighborhood of the two nuclei, but remain in the peripheral portion of the capsule, and later penetrate the eggs a short time before the beginning of maturation.

4. *Maturation.*—As in the case of all ova accompanied by the process of fertilization, those of *Graffilla* throw off two polar

bodies. The first maturation follows immediately upon the fusing of the chromosome vesicles produced by the aborting spindle, and at the time it occurs the sperm is already present in the egg (Fig. 21). The demonstration of maturation as taking place simultaneously in the two eggs within the same capsule is the most cogent proof we can offer against the idea that this animal exhibits polyembryony; because if this is a fact, each egg must subsequently be fertilized before it could develop, and that would at once remove the case from the category of polyembryony; and even though no other proof could be offered, such as we have given in connection with the section on the formation of the capsule, this would be sufficient to establish our main contention. As a matter of fact we have found two very clear cases in which each of the two eggs is undergoing maturescence.

The egg in one of these shows the first maturation spindle in the anaphase (Fig. 20). The spindle is extremely large and has at each end a large aster with very conspicuous centrospheres, in the lower of which is a single centrosome and in the upper of which are two centrosomes. The sperm head, already showing signs of its transformation into a pronucleus, lies near the lower aster. Between the upper pole of the spindle and the egg-membrane is a clear space due to a depression in the egg at this point. In a slightly later stage the egg elongates in the direction of the long axis of the spindle, taking on an appearance much like that of a pear, with the smaller end representing the animal pole. A very large polar body is then cut off, and the mate to this egg fortunately shows this process going on (Fig. 24). Since the first cleavage division results in producing a micromere of about the same size, opportunity is afforded for confusing this cell with the first polar body, but the difference can easily be told if the chromosomes are in a condition that allows their enumeration to be made.

In the second case one of the eggs (Fig. 22, on the left) shows the maturation spindle in prophase with four distinct tetrads, and the other cell a polar view, in which only three chromosomes appear. I have been unable to find a fourth tetrad, and I therefore assume that it must have been destroyed by the knife.

Several eggs showing the first polar body just extruded have been found. In a few of these the egg nucleus is in a resting condition, thus indicating that the second division may not follow immediately upon the first. However, I have not yet succeeded in finding the spindle of the second polar body division, but that a second polar body is thrown off is clearly shown in at least one case (Fig. 25). Here the constriction of the second polar body has just been completed, while the first polar body having undergone division is in the process of disintegration. The rapid disappearance of the polar bodies immediately after they are given off has added to the difficulty of studying their formation, as well as to the study of the formation of the first micromere.

Perhaps the most striking feature of maturation on *Graffilla* is the large size of the first polar body. This is not surprising; for it is not uncommon for a large polar body to be given off in the eggs of certain flat worms. It was in the egg of a turbellarian, *Prosthecerœus*, that Francotte, '97, discovered the interesting fact that the first polar body may be nearly as large as the egg itself, and may occasionally be fertilized and develop into a small gastrula, after having first formed a small polar body like the second one of the egg.

5. *Fertilization and the First Cleavage.*—Fertilization follows almost immediately upon the throwing off of the second polar body. I have found no exceptions to the rule that only one spermatozoön enters the egg. The sperm penetrates the egg in the vegetative hemisphere (Figs. 20, 21, 24), and passes toward the center where it remains while the polar bodies are being given off. The sperm nucleus then moves to a point near the animal pole where the copulation of the two pronuclei occurs (Fig. 26).

The first cleavage is unequal and results in cutting off a micromere at the animal pole. Any number of first cleavage spindles have been observed, and they are all characterized by having eight chromosomes, and by having centrosomes which are much more conspicuous than those of the maturation spindles. In this as in all of the subsequent early cleavages, the nuclei enter into a "rest" stage immediately after the completion of the division; and instead of forming a single vesicle, the chromosomes more or less retain their individuality, thus producing a number of small vesicles, some of which may, however, fuse together (Fig. 8).

IV. Some General Conclusions.

We find no evidence in *Graffilla* that the two embryos commonly found within a capsule are the product of a single fertilized egg. On the contrary, it is clear that they spring from two ova, which have become enclosed within a common envelope. In this respect our species does not present anything unusual; for while it is the rule among the rhabdocœles to have one embryo in a capsule, yet there are a number of well-known exceptions to this. In his excellent monograph on the turbellaria Von Graff, '08, has recently given a list (p. 2338) of these exceptions, which are as follows: *Gyratrix hermaphroditus*, *Provortex*, *Collastoma*, *Umagilla*, *Polycystis*, *Fecampia*, and *Monocelis lineata*, each has two embryos in a capsule; *Anoplodium*, 1–2; *Prorhynchus stagnalis*, 1–3; *P. balticus*, 6; *Graffilla*, 2–3; *Promesostoma marmoratum*, 4–7; *Dalyellia truncata*, *millportiana* and *viridis*, 4–12; *Plagiostomum vittatum* and *girardi*, 10–12; and finally, *Syndesmis*, 2–13. All of this goes to show that the facts which we have brought forward concerning the method of reproduction in *G. gemellipara* are entirely in harmony with what is known to occur in the other turbellaria. Even the manner in which the two ova become surrounded by nurse cells within the reproductive glands presents nothing new (unless it be in those cases in which the ova for a while lose their individuality). Furthermore, the habit of directly freeing the ova, with their nurse cells, into the mesenchyme is also seen in such forms as *Dalyellia viridis* and *Olisthanella obtusa*. In most forms in which two or more eggs are enclosed within a capsule the ova become surrounded by a common follicle of nurse cells before they pass to the uterus, where the shell or true capsule is usually secreted.

Some of the rather rare conditions seen in *G. gemellipara* are the indefiniteness of the reproductive ducts, the rudimentary state of the receptaculum seminis, the failure of the eggs to enter the uterus, and consequently the probable secretion of the shell by the mesenchyme. But all of these conditions are incident to the viviparous mode of reproduction. Linton suggests that this viviparity may be seasonal and parallel with the production of summer eggs, as is known to be the case in some of the Mesostomata. Certain facts in *Graffilla* might seem to indicate that what we have described are the conditions peculiar to a period of summer egg production. Thus the thin shell is a distinctive characteristic of a typical summer egg (Subitaneier), and the well developed unicellular shell-glands suggest at least that these organs could function later, if the species entered upon a period of winter egg production (Dauereier). However, in the absence of any proof that winter eggs are produced, and in the light of the fact that several of the female reproductive organs show a rudimentary or degenerate condition, we are inclined to believe that what we have described is the exclusive method of reproduction in this species. The presence of shell-glands, of a rudimentary receptaculum seminis, and of an indefinite uterus and ducts, instead of indicating that the species could later produce winter eggs, may and probably do, signify the close relationship of this species to the other members of the genus in which these structures are functional.

Of the half dozen species of *Graffilla* described in the literature, *G. gemellipara* appears to come closest, in its general arrangement of organs, to *G. Muricicola*. It also shows some similarity to *G. buccinicola*, but differs primarily from the latter in having the genital pore situated further back on the body.

In conclusion, we should like to point out, as a result of our studies on this animal, the necessity of exercising great precaution in concluding that a given species exhibits polyembryony. Undoubtedly the phenomenon of polyembryony will, in the future, be found to be much more extensive than we have suspected; but before coming to any definite conclusions, the investigator should trace the development back to the fertilized egg.

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PLATE I.

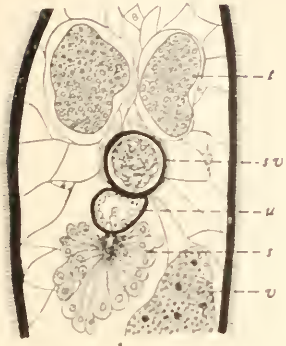
FIG. 1. Horizontal section of a young specimen, showing the testes (*t*), seminal vesicle (*sv*) which contains sperms, uterus (*u*), unicellular shell-glands (*s*), and the germ-vitellarium (*v*). × 222.

FIG. 2. Anterior half of a slightly oblique section from an adult individual. The uterus shows a distinct, but small diverticulum (*sr*) which in all probability corresponds to the receptaculum seminis of the other members of the genus. Note that the testes have disappeared. × 222.

FIG. 3. Horizontal section passing just below the intestine of a sexually matured individual. The section passes through the distal or bifurcated region of the uterus (*u*), which contains spermatozoa. *o*, ovary; *c*, capsule containing two eggs, one of which is giving off the first polar body; *v*, vitellarium. × 222.

FIG. 4. Horizontal section of another sexually matured animal, but which passes at a slightly lower level than the preceding. It shows clearly the bifurcated region of the uterus; and also the relationship existing between the uterus, ovary and vitellarium. × 222.

FIG. 5. A longitudinal median section (slightly schematized) of a rather old individual. It shows an advanced stage of the "female" condition. *m*, mouth; *ph*, pharynx; *oe*, œsophagus; *a*, atrium; *g*, genital pore; *p*, penis; *sv*, seminal vesicle; *s*, unicellular shell-glands; *u*, uterus; *v*, vitelline cell in uterus; *c*, capsules containing embryos; *i*, intestine. × 117.



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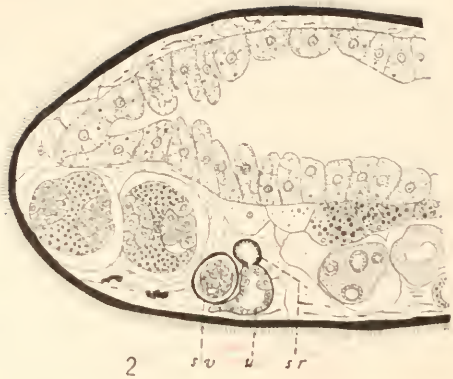

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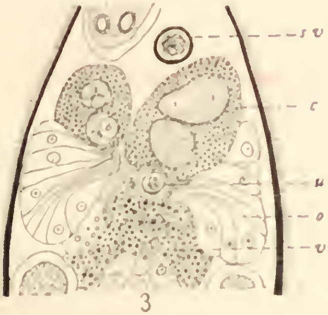

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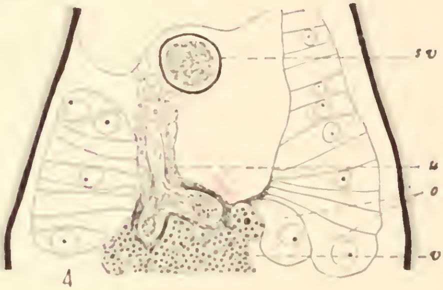

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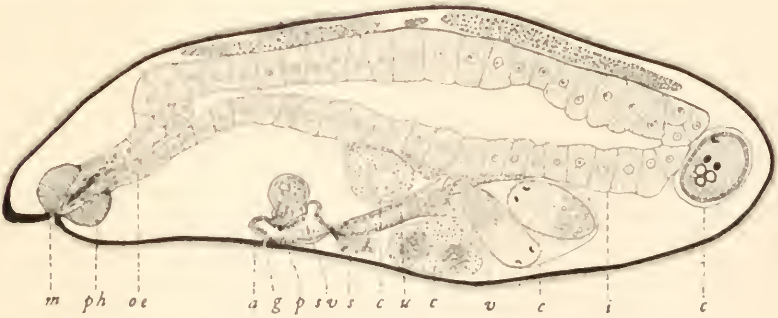
m
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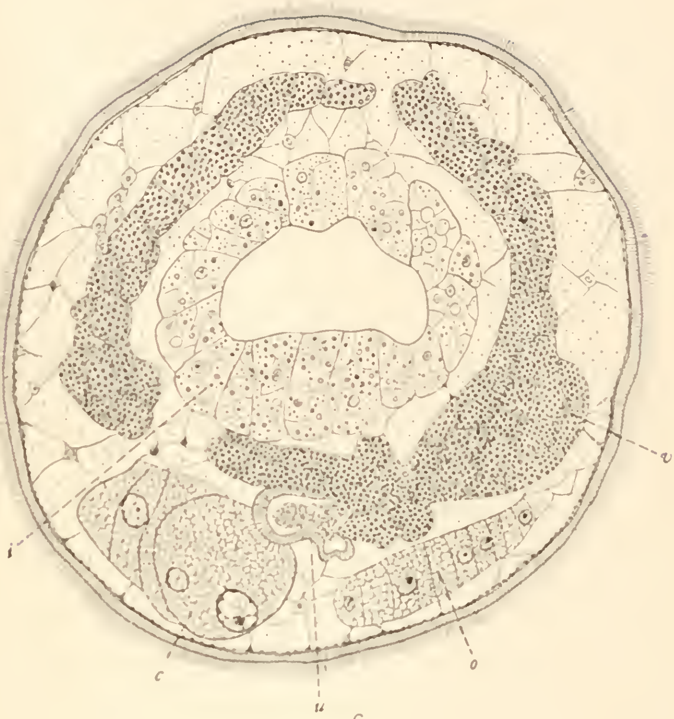
Plate II.

Fig. 6. Transverse section taken through the region of the tip of the uterus. × 381.

Fig. 7. Two ova that are beginning to be surrounded by vitelline cells preparatory to the formation of a capsule. × 784.

Fig. 8. The two-celled stage, showing a micromere and a macromere. × 740.



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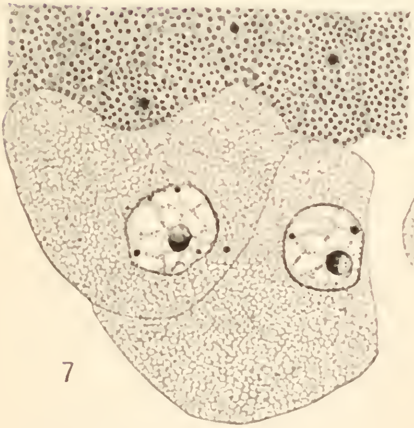

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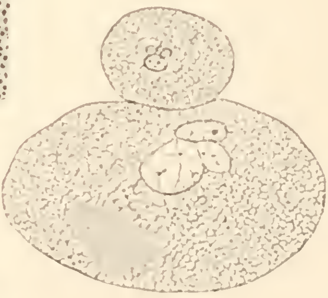

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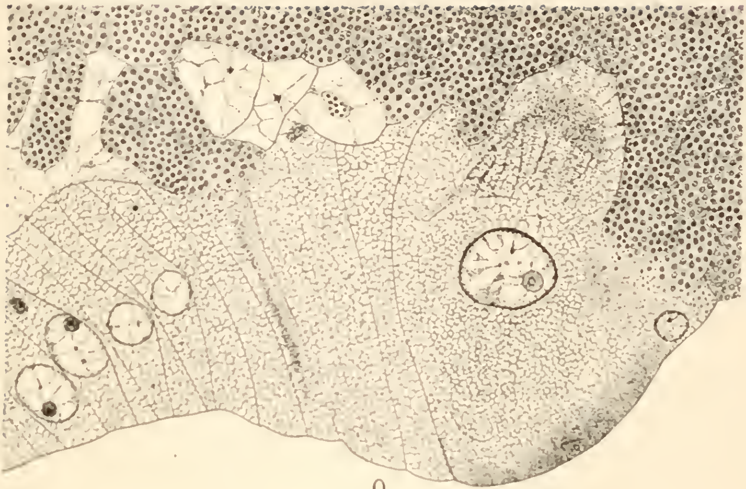
PLATE III.

FIG. 9. The posterior half of an ovary which shows the process of yolk absorption. On the right a capsule is being formed about two nuclei. × 650.

FIG. 10. A later stage in the same part of another ovary. Note that the two nuclei are immediately surrounded by a finely granular protoplasm. × 650.

FIGS. 11 and 12. Two eggs from the same capsule. This represents the condition shortly after the disappearance of the aborting spindle. The nucleus is in the form of faintly staining vesicles which in part are fused together. × 812.



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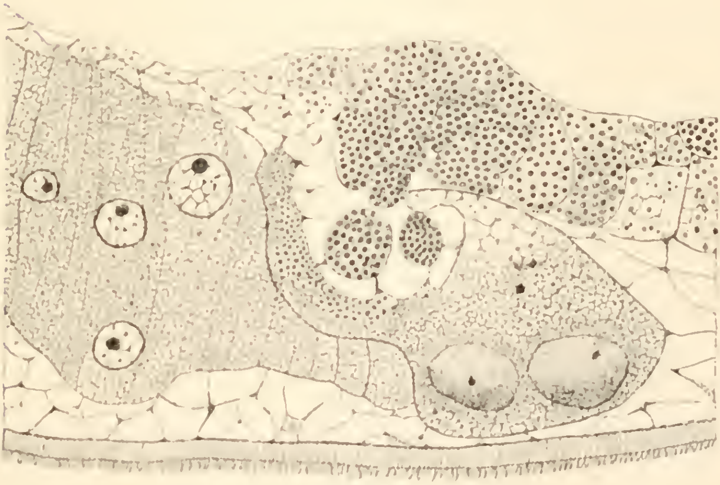

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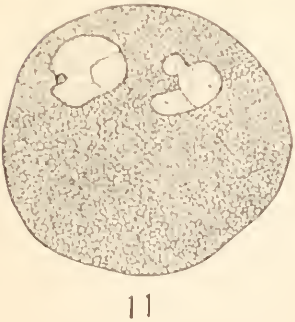

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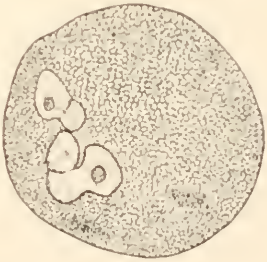

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PLATE IV.

FIG. 13. Two naked ova that have not become surrounded by a capsule. Such eggs apparently float about in the parenchyma, but probably never produce embryos. × 543.

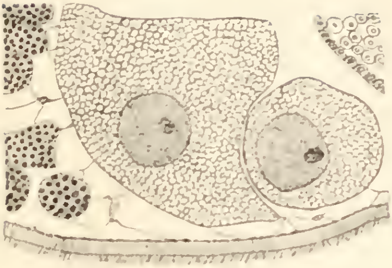
FIG. 14. A binucleated capsule in which the lower nucleus is surrounded by an intravitelline membrane. × 798.

FIG. 15. A binucleated capsule. × 543.

FIG. 16. Two ova completely surrounded by a follicular layer of vitelline cells. Only a part of one of the eggs is seen in the section. × 543.

FIG. 17. A typical case of an aborting spindle. Note that chromosomes are absent from the spindle. × 798.

FIG. 18. Another example of aborting spindle, in which the chromosomes are located among the rays at one end. × 798.



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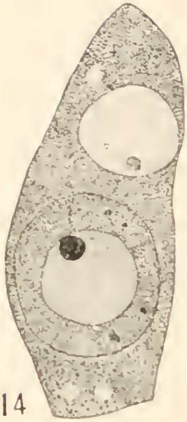

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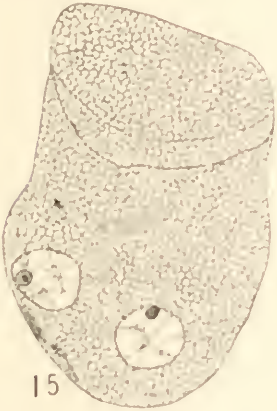

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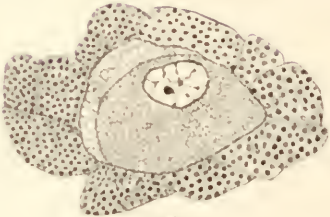

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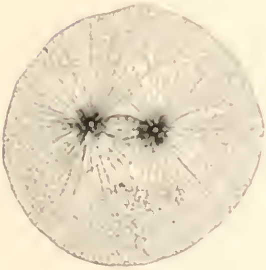

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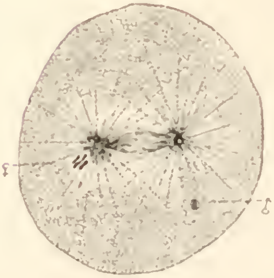

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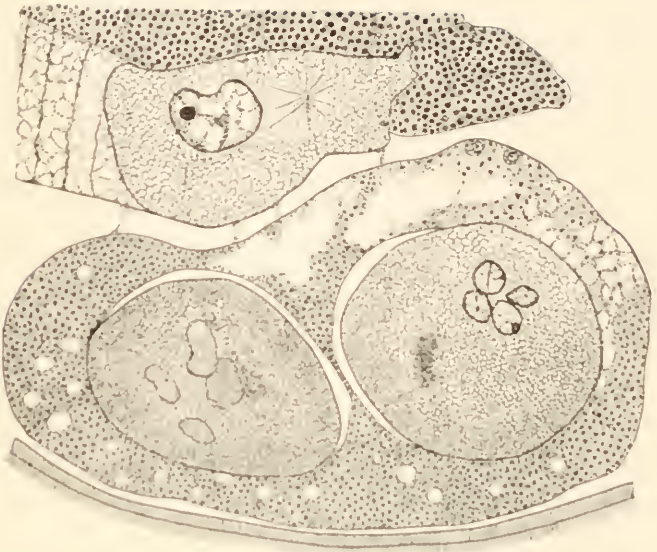
PLATE V.

FIG. 19. This shows a capsule about to be set free into the parenchyma. The eggs exhibit the condition which immediately follows the disappearance of the aborting spindle. Each egg has four chromosome-vesicles, and in the one on the left the centrosome is present. Lying just above this newly formed capsule is another in the process of formation. Only one of the ova shows in the section, and in it the centrosome has divided and the aster is present, thus anticipating the forthcoming aborting spindle. × 993.

FIG. 20. The anaphase stage of the first polar spindle. × 2,394.



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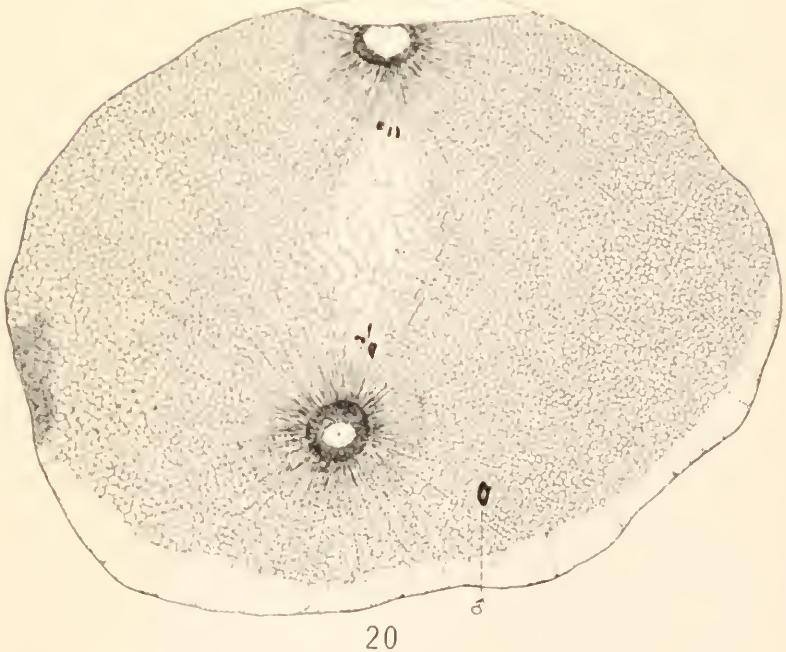

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Plate VI.

Fig. 21. An ovum shortly before the formation of the first polar body. The nucleus is the product of the fusion of the chromosome-vesicles of a stage like that in Fig. 19. The section passes through but one of the two ova in the capsule. In most of the capsules of this period the protoplasm of the eggs contracts in the reagents more than does the surrounding vitelline material, thus producing a clear space between the two materials. × 543.

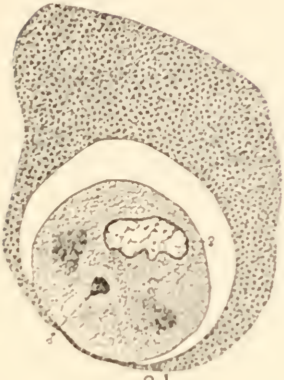
Fig. 22. A capsule in which both eggs are undergoing maturation at the same time. × 543.

Fig. 23. Two of the tetrads from the preceding figure. × 2,394.

Fig. 24. The cutting off of the first polar body. This egg is a mate to the one shown in Fig. 20. × 798.

Fig. 25. This stage shows the close of maturation. The first polar body has undergone division and is disintegrating. × 543.

Fig. 26. Fertilization stage. × 543.



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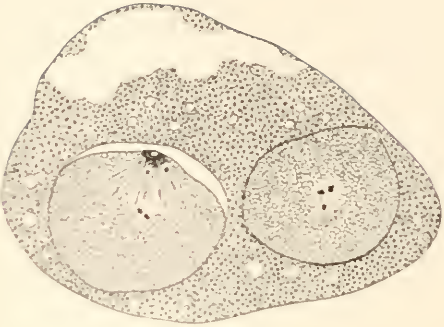

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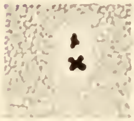

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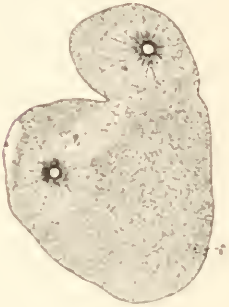

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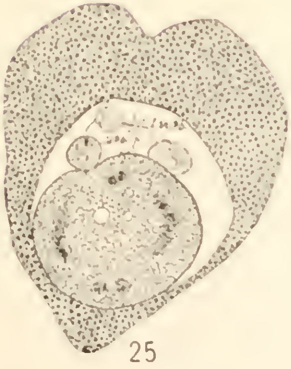

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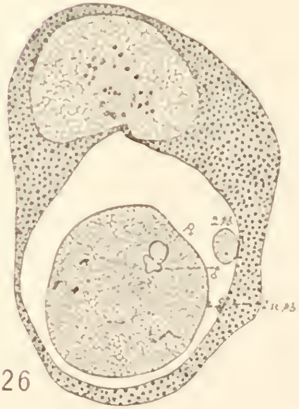

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J. T. PATTERSON

Vol. XXII. *March, 1912.* *No. 4*

BIOLOGICAL BULLETIN

"STRAINS" IN HYDATINA SENTA.

D. D. WHITNEY.

In a former paper results of experiments upon two races of the rotifer *Hydatina senta* were given in regard to the production of one hundred generations of females without the appearance of males in either race. These experiments have been extended further for about seventeen months and as they are concluded it seems desirable to record the results obtained partly as a confirmation of the earlier conclusions and partly because they form evidence which shows that there exists different races or strains or lines within this particular species of *Hydatina senta*.

In the former paper it was shown how readily male-producing females could be produced in newly made dilute uncooked horse manure cultures and also how readily the male-producing females could be repressed in newly made concentrated cooked horse manure cultures.

In the present paper the parallel history of three races of rotifers *A*, *B*, and *C* is given. Races *B* and *C* are the same races upon which the former conclusions were based while race *A* is an additional one. Races *A* and *B* are sister races, both having developed from one fertilized egg while race *C* is unrelated to races *A* and *B* except in as far as all three races came from the same general culture of rotifers which was originally collected at Grantwood, New Jersey, in 1906.

Races *A* and *B* were always conducted in a parallel series but race *C* was not put into the parallel series until it was in the 36th generation. During this early period of the three races before they were all put into the parallel series the food was from miscellaneous protozoa cultures of various ages made in dilute uncooked horse manure media. The summary of the early history of these three races before they were all conducted in the parallel series is recorded in Table I. The percentage of male-producing females of races *A* and *B* are practically equivalent, while that of race *C* is much lower.

TABLE I.

Showing the number of female- and male-producers in the three races *A*, *B*, and *C*, from their origin to the time at which parallel records were taken. Female-producers are designated ♀♀, male-producers ♂♀.

Race.	Generations.	No. of ♀♀.	No. of ♂♀.	Per Cent. of ♂♀.	Time.	Food.
A	1-144	1,188	181	13.22+	Oct. 6, 1908, to Aug. 31, 1909.	Dilute uncooked horse manure media, 7-28 days old. Miscellaneous protozoa growing in them.
B	1-146	1,224	167	12.00+	Oct. 6, 1908, to Aug. 31, 1909.	
C	1- 35	210	10	4.54+	June 16, 1909, to Aug. 31, 1909.	

September 3, 1909, these three races *A*, *B*, and *C* were started in a parallel series under as identical external conditions as possible. At the beginning of this parallel series the generations were renumbered and the beginning generation of each of the races in this series is called No. I. Ten young females from each

TABLE II.

Race *C*.

Generation.	No. of ♀♀.	No. of ♂♀.	Per Cent. of ♂♀.	Time.	Food.
1	9	3	25	June 16, 1909, June 18, 1909.	Same as Table I.
2	20	0	0	June 18, 1909, June 20, 1909.	
3	16	4	20	June 21, 1909, June 22, 1909.	
4	8	2	20	June 24, 1909, June 26, 1909.	
Partial summary.	53	9	14.51+		
5-34	150	0	0	June 26, 1909, Aug. 30, 1909.	
35	7	1	12.5	Aug. 30, 1909, Sept. 1, 1909.	
Total summary.	210	10	4.54+		

Detailed history of race *C* throughout the first 35 generations, which is also summarized in Table I.

generation of each race were isolated at the same time and each female placed in a Syracuse watch glass and allowed to mature and to produce daughter females. Then this process was repeated for 345 generations. All the females at each isolation were placed in the same quantity of tap water to which was added the same amount of food culture that was taken from one food jar. The watch glasses in which the rotifers lived always were in three stacks side by side at room temperature. Practically all external influences were as identical as it was possible to make them.

The detailed observations are given in Table III. in parallel columns and the summary is given in Table IV.

At the end of Table I., races *A* and *B*, which up to this time were fed on various protozoa cultures, were practically identical in regard to the percentages of male-producing females in each race, but at the beginning of Tables III. and IV. when the two races were subjected to uncooked concentrated food culture media a decided change occurred. Race *A* retained and even exceeded its former rate of production of male-producing females, but in race *B* the rate was very perceptibly lowered. Race *C* lowered slightly its rate of male-producing females. This occurred during the first 56 generations. From the 57th to the 345th generation in races *A* and *C* and to the end of race *B*, the 239th generation, concentrated cooked food media was used and caused a decided lowering of the production of male-producing females in all races. In race *C* this was reduced to zero, in race *B* to less than 1 per cent., and in race *A* to about 3.5 per cent.

This confirms the earlier results in showing that it is possible by external conditions to repress entirely the production of male-producing females in some races of this rotifer for a long period of time. In race *C* the male-producing females were repressed for 280 generations and then reappeared when the food media was made too dilute accidentally.

If these three races were exactly alike in their power to produce male-producing females and all were subjected to the same external conditions they ought to produce such male-producing females at the same rate. However, as the above observations show that the rates of production of male-laying females vary

TABLE III.

Showing number of female- and male-producers in a parallel series of 345 generations in the three races *A*, *B*, and *C*. Generations 1–56 show the detailed results when the three races were fed upon concentrated uncooked food media and generations 57–345 show the detailed results when the same three races were fed upon concentrated cooked media.

Generation.	Race *A*.		Race *B*.		Race *C*.	
	No. of ♀ ♀.	No. of ♂ ♀.	No. of ♀ ♀.	No. of ♂ ♀.	No. of ♀ ♀.	No. of ♂ ♀.
1	5	0	6	0	7	0
2	8	2	10	0	10	0
3	9	0	10	0	9	0
4	9	0	9	0	9	0
5	9	0	8	0	9	0
6	9	0	9	0	9	0
7	9	0	9	0	9	1
8	10	0	9	0	10	0
9	8	2	10	0	10	0
10	8	2	10	0	9	1
11	9	1	10	0	10	0
12	10	0	10	0	10	0
13	10	0	10	0	10	0
14	7	3	10	0	9	1
15	9	1	9	1	10	0
16	7	3	8	1	10	0
17	10	0	10	0	10	0
18	9	1	10	0	10	0
19	10	0	9	0	9	1
20	9	1	10	0	10	0
21	8	2	10	0	8	2
22	8	2	9	1	10	0
23	9	1	8	1	10	0
24	8	2	10	0	10	0
25	9	1	10	0	10	0
26	9	1	9	1	9	1
27	5	5	10	0	10	0
28	10	0	10	0	9	0
29	10	0	10	0	9	1
30	8	2	10	0	10	0
31	5	5	9	1	10	0
32	10	0	8	2	10	0
33	10	0	10	0	10	0
34	4	6	10	0	10	0
35	6	4	9	0	10	0
36	6	3	10	0	10	0
37	9	1	10	0	10	0
38	8	2	10	0	10	0
39	9	1	10	0	9	1
40	1	9	6	4	6	4
41	7	3	10	0	10	0
42	8	2	10	0	10	0
43	7	3	10	0	10	0
44	7	3	10	0	9	1
45	7	1	9	0	9	0
46	7	3	10	0	10	0
47	7	3	9	1	10	0

TABLE III.—*Continued.*

Generation.	Race *A*.		Race *B*.		Race *C*.	
	No. of ♀♀.	No. of ♂♀.	No. of ♀♀.	No. of ♂♀.	No. of ♀♀.	No. of ♂♀.
48	7	3	10	0	9	1
49	6	3	9	1	10	0
50	7	3	9	1	10	0
51	7	3	10	0	10	0
52	7	2	10	0	10	0
53	10	0	10	0	10	0
54	9	1	10	0	10	0
55	10	0	10	0	9	1
56	6	4	9	1	8	2
57	10	0	10	0	10	0[1]
58	10	0	10	0	10	0
59	8	2	10	0	10	0
60	4	6	10	0	10	0
61	8	2	10	0	10	0
62	4	6	10	0	10	0
63	7	2	10	0	10	0
64	10	0	10	0	10	0
65	10	0	10	0	10	0
66	9	1	10	0	10	0
67	8	2	10	0	10	0
68	10	0	10	0	10	0
69	10	0	10	0	10	0
70	10	0	10	0	10	0
71	5	3	8	0	10	0
72	10	0	10	0	10	0
73	10	0	10	0	10	0
74	10	0	10	0	10	0
75	10	0	10	0	10	0
76	10	0	10	0	10	0
77	10	0	10	0	10	0
78	10	0	10	0	10	0
79	10	0	10	0	10	0
80	10	0	10	0	10	0
81	10	0	10	0	10	0
82	9	1	10	0	10	0
83	10	0	10	0	10	0
84	10	0	10	0	10	0
85	10	0	10	0	10	0
86	10	0	10	0	10	0
87	10	0	10	0	10	0
88	10	0	10	0	10	0
89	10	0	10	0	10	0
90	10	0	10	0	10	0
91	9	1	10	0	10	0
92	10	0	10	0	9	0
93	10	0	10	0	10	0
94	10	0	10	0	10	0
95	10	0	10	0	10	0
96	10	0	10	0	10	0
97	10	0	10	0	10	0
98	10	0	10	0	10	0
99	10	0	10	0	10	0

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[1] Concentrated cooked horse manure media began to be used.

TABLE III.—*Continued.*

Generation.	Race *A.*		Race *B.*		Race *C.*	
	No. of ♀♀.	No. of ♂♀.	No. of ♀♀.	No. of ♂♀.	No. of ♀♀.	No. of ♂♀.
100	9	1	10	0	10	0
101	10	0	10	0	10	0
102	10	0	10	0	10	0
103	10	0	10	0	10	0
104	10	0	10	0	10	0
105	9	1	10	0	10	0
106	10	0	10	0	10	0
107	10	0	10	0	10	0
108	10	0	10	0	10	0
109	10	0	10	0	10	0
110	10	0	10	0	10	0
111	10	0	10	0	6	0
112	10	0	10	0	10	0
113	10	0	10	0	10	0
114	10	0	10	0	10	0
115	10	0	10	0	10	0
116	10	0	10	0	10	0
117	10	0	10	0	10	0
118	10	0	10	0	10	0
119	10	0	10	0	10	0
120	10	0	10	0	10	0
121	10	0	10	0	10	0
122	10	0	10	0	10	0
123	10	0	10	0	10	0
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138	10	0	10	0	10	0
139	10	0	10	0	10	0
140	10	0	10	0	10	0
141	10	0	10	0	10	0
142	10	0	10	0	10	0
143	10	0	10	0	10	0
144	10	0	10	0	10	0
145	10	0	10	0	10	0
146	10	0	10	0	10	0
147	10	0	10	0	10	0
148	10	0	10	0	10	0
149	10	0	10	0	10	0
150	10	0	10	0	10	0
151	10	0	10	0	10	0
152	10	0	10	0	10	0
153	10	0	10	0	10	0

TABLE III.—*Continued.*

Generation.	Race *A*.		Race *B*.		Race *C*.	
	No. of ♀♀.	No. of ♂♀.	No. of ♀♀.	No. of ♂♀.	No. of ♀♀.	No. of ♂♀.
154	10	0	10	0	10	0
155	10	0	10	0	10	0
156	10	0	10	0	10	0
157	10	0	10	0	10	0
158	10	0	10	0	10	0
159	10	0	10	0	10	0
160	10	0	10	0	10	0
161	10	0	10	0	10	0
162	10	0	10	0	10	0
163	10	0	10	0	10	0
164	10	0	10	0	10	0
165	10	0	10	0	10	0
166	9	1	10	0	10	0
167	9	1	10	0	10	0
168	10	0	10	0	10	0
169	10	0	10	0	10	0
170	10	0	10	0	10	0
171	10	0	10	0	10	0
172	10	0	10	0	10	0
173	10	0	10	0	10	0
174	10	0	10	0	10	0
175	10	0	10	0	10	0
176	10	0	10	0	10	0
177	10	0	10	0	10	0
178	10	0	10	0	10	0
179	10	0	10	0	10	0
180	10	0	10	0	10	0
181	10	0	10	0	10	0
182	10	0	10	0	10	0
183	10	0	10	0	10	0
184	10	0	10	0	10	0
185	10	0	10	0	10	0
186	10	0	10	0	10	0
187	10	0	10	0	10	0
188	10	0	10	0	10	0
189	10	0	10	0	10	0
190	8	2	10	0	10	0
191	10	0	10	0	10	0
192	10	0	10	0	10	0
193	2	0	2	0	1	0
194	1	0	1	0	1	0
195	4	0	10	0	10	0
196	6	0	8	0	3	0
197	10	0	10	0	10	0
198	10	0	10	0	10	0
199	10	0	10	0	10	0
200	10	0	10	0	10	0
201	10	0	10	0	10	0
202	10	0	10	0	10	0
203	9	1	10	0	10	0
204	9	1	10	0	10	0
205	10	0	10	0	10	0
206	10	0	10	0	10	0
207	10	0	10	0	10	0

Table III.—*Continued.*

Generation.	Race *A*.		Race *B*.		Race *C*.	
	No. of ♀♀.	No. of ♂♀.	No. of ♀♀.	No. of ♂♀.	No. of ♀♀.	No of ♂♀.
208	10	0	10	0	10	0
209	10	0	10	0	10	0
210	10	0	10	0	10	0
211	10	0	10	0	10	0
212	10	0	9	1	10	0
213	10	0	10	0	10	0
214	10	0	10	0	10	0
215	10	0	10	0	10	0
216	8	0	8	0	8	0
217	9	1	10	0	10	0
218	10	0	10	0	10	0
219	10	0	10	0	10	0
220	10	0	10	0	10	0
221	10	0	10	0	10	0
222	5	0	4	0	10	0
223	10	0	10	0	10	0
224	10	0	10	0	10	0
225	10	0	10	0	10	0
226	10	0	9	1	10	0
227	1	0	1	0	1	0
228	10	0	10	0	10	0
229	10	0	10	0	10	0
230	10	0	4	6	10	0
231	10	0	10	0	10	0
232	10	0	5	0	10	0
233	10	0	10	0	10	0
234	7	0	4	0	9	0
235	1	0	2	0	10	0
236	4	0	1	0	3	0
237	10	0	1	0	10	0
238	10	0	4	0	10	0
239	10	0	0	0	9	0
240	10	0	Died.		10	0
241	3	0			10	0
242	4	0			10	0
243	10	0			10	0
244	8	2			10	0
245	10	0			10	0
246	10	0			10	0
247	10	0			10	0
248	10	0			10	0
249	10	0			10	0
250	10	0			10	0
251	10	0			10	0
252	9	0			10	0
253	10	0			10	0
254	9	0			9	0
255	10	0			10	0
256	6	0			9	0
257	10	0			10	0
258	10	0			10	0
259	10	0			10	0
260	10	0			10	0
261	10	0			10	0

TABLE III.—*Continued.*

Generation.	Race *A*.		Race *B*.		Race *C*.	
	No. of ♀♀.	No. of ♂♀.	No. of ♀♀.	No. of ♂♀.	No. of ♀♀.	No. of ♂♀.
262	7	0			10	0
263	9	0			10	0
264	9	0			10	0
265	10	0			10	0
266	10	0			10	0
267	10	0			10	0
268	10	0			10	0
269	10	0			10	0
270	10	0			10	0
271	10	0			10	0
272	10	0			10	0
273	10	0			10	0
274	7	3			10	0
275	10	0			10	0
276	10	0			10	0
277	10	0			10	0
278	10	0			10	0
279	10	0			10	0
280	8	0			10	0
281	9	1			10	0
282	8	2			10	0
283	8	2			10	0
284	10	0			10	0
285	4	6			10	0
286	5	4			10	0
287	9	1			10	0
288	8	2			10	0
289	6	0			10	0
290	7	0			7	0
291	6	1			9	0
292	8	0			2	0
293	9	0			10	0
294	10	0			10	0
295	4	0			9	0
296	4	0			9	0
297	1	0			9	0
298	4	1			9	0
299	4	5			4	0
300	8	0			8	0
301	7	0			10	0
302	10	0			10	0
303	9	0			8	0
304	10	0			10	0
305	9	0			8	0
306	10	0			8	0
307	8	0			9	0
308	4	0			7	0
309	6	0			10	0
310	7	0			10	0
311	10	0			10	0
312	5	0			5	0
313	5	0			5	0
314	5	0			5	0
315	3	2			5	0

TABLE III.—*Concluded.*

Generation.	Race *A*.		Race *B*.		Race *C*.	
	No. of ♀♀.	No. of ♂♀.	No. of ♀♀.	No. of ♂♀.	No. of ♀♀.	No. of ♂♀.
316	4	1			5	0
317	5	0			5	0
318	5	0			5	0
319	10	0			10	0
320	8	2			10	0
321	5	0			5	0
322	5	0			5	0
323	4	1			5	0
324	5	0			5	0
325	5	0			10	0
326	9	1			10	0
327	6	2			10	0
328	3	0			10	0
329	9	1			10	0
330	10	0			10	0
331	7	3			10	0
332	10	0			10	0
333	8	2			10	0
334	6	4			10	0
335	8	0			10	0
336	8	2			10	0
337	8	2			10	0
338	9	1			10	0
339	10	0			10	0
340	10	0			10	0
341	10	0			10	0
342	10	0			10	0
343	4	2			10	0
344	9	0			10	0
345	5	1			10	0
346	6	0			8	2[1]
347	8	0			10	0
348	5	0			9	1
349	3	0			9	1

in the three strains, *A*, *B*, and *C*, when all external conditions are identical the only conclusion that can be drawn is that the three strains differ at least in regard to this single characteristic. Perhaps they all may be potentially alike in their capacity to produce male-laying females but some races may be more easily effected than other races by the influence which causes male-producing females to be produced.

Whenever races *A* and *B* were put into newly made diluted uncooked culture media in battery jars great numbers of fertilized eggs were produced in both races. From general observations they seemed to be produced in equal numbers, thus seeming to

[1] Food media was diluted accidentally.

form evidence that these two races were potentially alike in their power to produce male-producing females but when conditions were unfavorable they differed, as shown in the parallel series, in their responsiveness to the influences that so acted upon the females as to cause them to produce male-producing daughter females. However, when race *C* was put into newly made uncooked culture media in battery jars very few fertilized eggs were produced, thus seeming to show either that this race *C* was potentially different from the other two races in its capacity to produce male-producing females or that it was not as easily acted upon by the male-producing female influences as were races *A* and *B*. Notwithstanding this fact that race *C* produced very few male-producing females when put into battery jars containing dilute uncooked horse manure media it should be stated that in the early history of race *C* it had as high a percentage of male-producing females in the first four generations as was found in either race *A* or *B*, Table II. The race was isolated from a general culture jar in which an abundance of males were appearing at the time of isolation. Beginning with generation five very few males appeared thereafter. This early history shows that race *C* at one time was as potential in its power to produce male-producing females as races *A* and *B*, but whether it later lost this power or never was again subjected to as favorable influences for the production of male-producing females it is impossible to state. Whatever may be the true explanation of this divergence in the male-producing female rates of the three races it surely indicates a difference in the races either in their capacity to produce male-producing females or their responsiveness to the influences that cause male-producing females to be produced.

Punnett concluded that he found "sex strains" in *Hydatina senta* which differed in their power to produce males and even concluded that he found some strains that produced no males. It is very possible that such maleless strains were really like race *C* in the above experiments. From observations and experiments published in an earlier paper ('07), it was shown that no pure female strains could be found. The results of the present experiments corroborate this earlier conclusion. However, the evidence at that time showed no strains of any kind but the

TABLE IV.

Summary of Table III. showing the influence of concentrated uncooked horse manure media, containing various protozoa and concentrated cooked horse manure media containing only the protozoa, *Polytoma*.

Generations.	Race *A*.			Race *B*.			Race *C*.			Time.	Food.
	No. of ♀♀.	No. of ♂♀.	Per Cent. of ♂♀.	No. of ♀♀.	No. of ♂♀.	Per Cent. of ♂♀.	No. of ♀♀.	No. of ♂♀.	Per Cent. of ♂♀.		
1–56	445	100	18.34+	529	16	2.93+	533	18	3.26+	Sept. 3, 1909, to Jan. 16, 1910.	Uncooked concentrated horse manure media, 2–5 days old. *Polytoma* and other protozoa.
57–345	2,565	92	3.46+				2,749	0	0	Jan. 16, 1910, to Nov. 15, 1911.	
57–238				1,731	8	0.45+				Jan. 16, 1910, to Mar. 6, 1911.	Cooked concentrated horse manure media, 1½–3 days old. Pure culture of *Polytoma*.

present evidence collected from observations extending over a period of about three years and including 300–500 parthenogenetic generations shows very clearly that strains exist which differ in their percentage of male-producing females.

Moreover, the two sister strains *A* and *B* which developed from the same fertilized egg differed in their longevity. Strain *B* died out from general exhaustion in the 384th parthenogenetic generation, while strain *A* is still alive in the 504th parthenogenetic generation, although in a very weak and exhausted condition.

Shull has compared some of the New York strains of *Hydatina senta* with a strain from Baltimore and has found a decided difference in the rate of production of males in the two strains. He says, "It is safe to say, therefore, that we have here two pure lines that differ from one another in a fairly constant manner, and the difference is an internal one."

SUMMARY.

1. The production of male-producing females can be partly or wholly repressed by external conditions in parthenogenetic races of *Hydatina senta*.

2. The parthenogenetic strains are shown to be distinct because under identical external conditions they differ in their power to produce male-producing females. This may indicate that they differ in their potentiality of producing male-producing females or that they differ in degree of responsiveness to the influences which cause male-producing females to be produced. The latter alternative seems more probable.

3. The two sister parthenogenetic strains developing from one fertilized egg differed in their longevity. One lived about a year longer and produced over one hundred more generations than the other.

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MIDDLETOWN, CONN.,
January 3, 1912.

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SUPERNUMERARY CHROMOSOMES, AND SYNAPSIS IN CEUTHOPHILUS (SP.?).

N. M. STEVENS.

The species of *Ceuthophilus* which I have used in this study, I have not been able to identify. The material seems to be homogeneous, and is the only species of this genus that I have seen about Bryn Mawr. The insects were found, usually in pairs, in their burrows under stones, and were collected in October and November, 1910 and 1911. They are not abundant, and only 7 males were secured in 1910, and 5 in 1911.

METHODS.

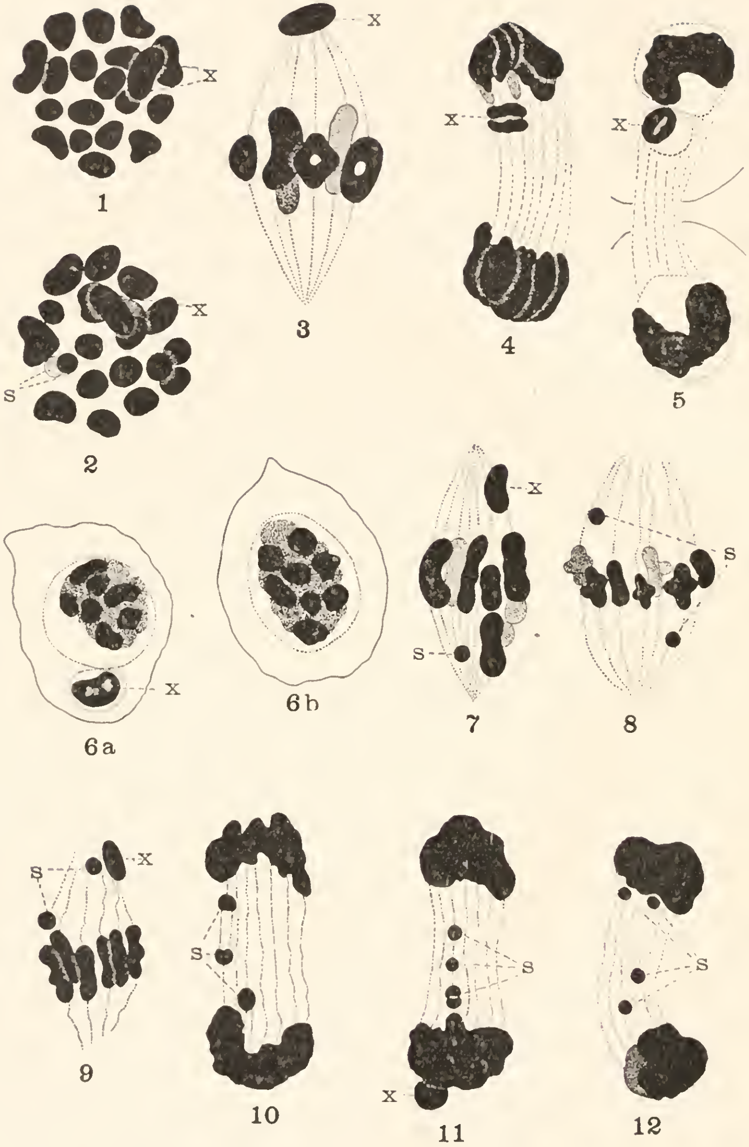
In the case of each individual a few aceto-carmine preparations were made, and the remainder of the testes fixed in Gilson's mercuro-nitric fluid or in Flemming's stronger chrom-osmic-acetic mixture. The best results were obtained from sections of Flemming material, cut 10μ thick and stained with thionin.

SUPERNUMERARY CHROMOSOMES.

In one of the 1910 insects one, and in another two supernumerary chromosomes were found. These chromosomes are readily distinguished from the other chromosomes by their smaller size and erratic behavior.

The spermatogonial chromosomes, like those of *Stenopelmatus*, are difficult to count, because they do not form a flat plate at any stage, but lie at somewhat different levels and overlap. The number is probably 37, exclusive of supernumeraries.

In the first spermatocytes there are 18 bivalents and the univalent X (19), when no supernumeraries are present (Fig. 1). Fig. 2 shows 18 bivalents, the unpaired chromosome X, and 2 supernumeraries (s). The odd chromosome X is usually found at one pole of the spindle when the other chromosomes are in metaphase (Fig. 3), but it not infrequently lags behind the others in the anaphase (Fig. 4) and is enclosed in a separate membrane


x
1
x
s
2
x
3
x
4
x
5
x
6a
6b
x
s
7
s
8
s
x
9
s
10
s
x
11
s
12

Figs. 1–2. Metaphase of first maturation mitosis, showing $18+X$ and $18+X+2s$. (Mag. 1,500 for all figures.)

Figs. 3–5. Metaphase and anaphases showing position of X.

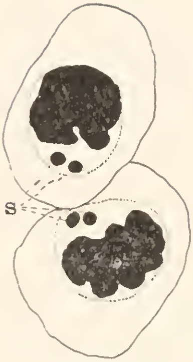
Figs. 6*a* and *b*. Young sister spermatocytes of the second order.

Figs. 7–12. Variations in position and division of the two supernumeraries in first spermatocytes.

in the telophase (Fig. 5). Figs. 6*a* and 6*b* are sister spermatocytes of the second order, showing *X* in a separate vesicle; this does not happen in by any means one half of the second spermatocytes, *X* being, I should say, more often included within the same nuclear membrane with the other chromosomes.

Fig. 7 is from the individual which had one supernumerary, and Figs. 8 to 14 from the one that had two. Fig. 8 also shows a less usual position of *X*, near the equatorial plate. As in the *Diabroticas* (Stevens, '08) the supernumeraries may or may not divide in the first maturation mitosis, and they may, when undivided, go to either pole of the spindle. Their position in the metaphase does not necessarily indicate whether they will divide or not. The determining factor is probably the attachment of spindle fibers from one or from both poles. The supernumeraries in Figs. 7, 8 and 9 would not divide in this mitosis, but the presence of a supernumerary in an equatorial plate or between daughter plates does not necessarily assure its division. In Fig. 10 one is divided, the other undivided, while in Figs. 11 and 12 both are divided. In the telophases shown in Figs. 13 and 14, both supernumeraries are divided in one case and neither in the other. The possible combinations of *X* and the 2 supernumeraries in the spermatids are *X*, *X* + 1*s*, *X* + 2*s*, 0, 1*s* and 2*s*. Four of the possibilities are shown in Figs. 15 to 18.

As to the origin of these supernumeraries, there is little evidence in this material. In *Metapodius* Wilson ('09) discovered the probable origin of the supernumeraries in an irregular second spermatocyte mitosis in which both "idiochromosomes" went to the same pole of the spindle, and therefore to the same spermatid. The supernumeraries are thus shown to be duplicates of the smaller "idiochromosome" in *Metapodius*, or in one case of an "*m*-chromosome" ('10). In neither *Diabrotica* nor in *Ceuthophilus* is there a smaller mate for the *X* chromosome present. The three supernumeraries which have been observed in *Ceuthophilus* are of about the same size; considerably less than one half, and apparently about one fourth the size of *X*. The behavior of the supernumeraries in growth and rest stages of the nucleus indicates their probable relationship to *X*, and their behavior in mitosis, dividing only once, either in the first or the second



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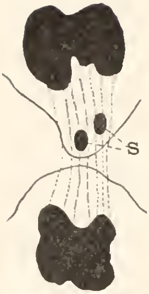

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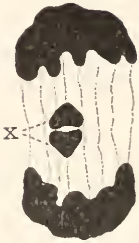

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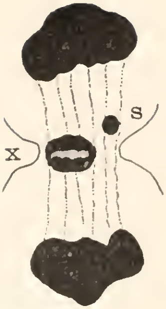

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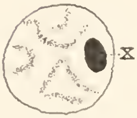

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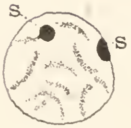

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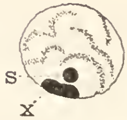

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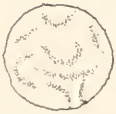

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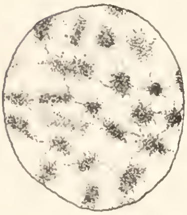

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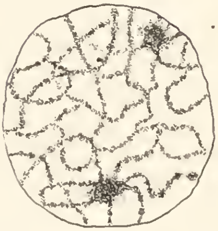

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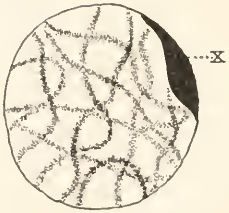

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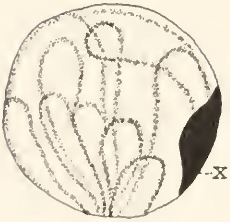

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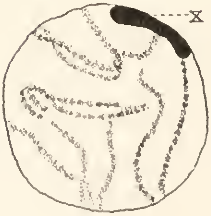

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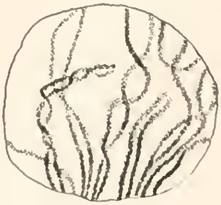

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FIGS. 13–14. Telophases showing the supernumeraries divided (13) or undivided (14).

FIGS. 15–18. Spermatid nuclei showing variations as to presence or absence of X and the two supernumeraries.

FIGS. 19–20. Anaphases showing unusual position of X.

maturation mitosis, shows that they are univalent. In *Diabrotica soror* I have considerable evidence that the supernumeraries owe their origin to a transverse and a longitudinal division of *X* ('12), and it seems probable that those of *Ceuthophilus* have had a similar origin. I have occasionally found cases where *X* seemed about to divide late in the first maturation mitosis (Figs. 19 and 20), but I have as yet no evidence of a transverse division.

SYNAPSIS.

The material which was collected in 1911 with the hope of getting more light on the origin of the supernumeraries, proved to be favorable for a study of synapsis, or, as I should prefer to call the phenomenon, conjugation of the chromosomes. These testes were all fixed in Flemming and stained with either thionin or iron-haematoxylin. Thionin gave the clearest figures.

In the resting nuclei of the spermatogonia the chromosomes are either visible as separate individuals as in Fig. 21, or are more or less completely resolved into rather fine spireme threads as in Fig. 22. In some follicles one sees only such spermatogonial nuclei as in Fig. 21; in others the various cysts show various degrees of resolution into spireme threads. The former condition I should attribute to more rapid division of the spermatogonia, the time between mitoses being insufficient for complete resolution.

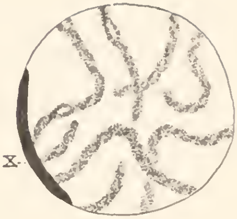
In the youngest spermatocytes, distinguished from the spermatogonia by the condensed condition of *X*, the spireme threads are similar to those of the spermatogonia, perhaps a little coarser. They are finely granular and more or less nodular. There is no contraction, or synizesis, stage and no complete polarization of loops to form a perfect bouquet stage. The spireme threads are usually irregularly but rather evenly distributed through the nucleus as in Fig. 23, which also shows *X* in characteristic position against the nuclear membrane. Fig. 24 shows an extreme and

FIGS. 21–22. Spermatogonial nuclei, showing resolved and unresolved chromosomes.

FIG. 23. Spermatocyte nucleus before synapsis.

FIG. 24. Similar stage showing partial polarization of chromosomes.

FIGS. 25–26. Stages in parasynapsis.



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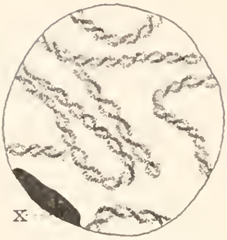

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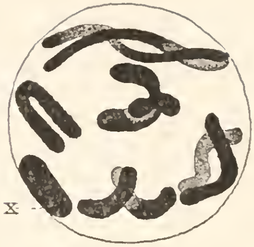

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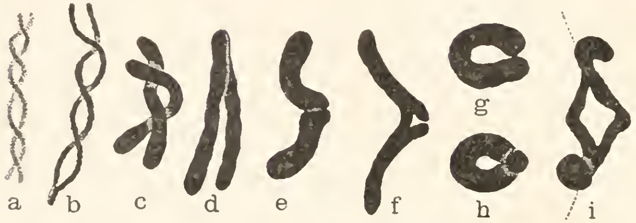

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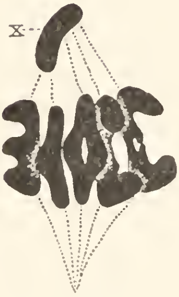

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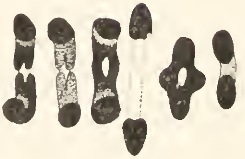

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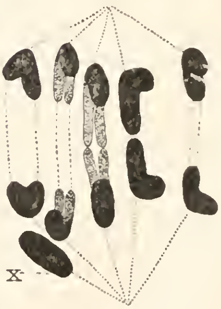

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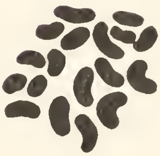

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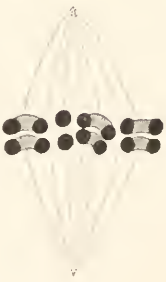

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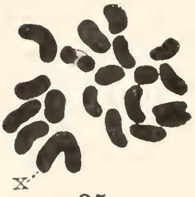

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FIG. 27. Double spireme stage.

FIGS. 28–29. Prophase stages.

FIG. 30*a*–*i*. Various prophase (*a–g*) and metaphase (*h*, *i*) forms of the bivalent chromosomes.

FIGS. 31–32. Chromosomes in metaphase of the first maturation mitosis.

unusual case of polarization, but here only nine polarized loops are present and the other spireme threads extended in various directions through the nucleus. In some follicles there is a perfectly sharp division-line between cysts containing spermatocyte nuclei of the character of that shown in Fig. 23, and cysts in which the spireme is about twice as thick (Fig. 27). There is no evidence of telosynapsis, and also no evidence of any such longitudinal contraction of the threads as would be required to give the thicker spireme of Fig. 27. In other follicles there come in between these two stages, other cysts in which as a rule the chromatin threads are very irregularly distributed; *i. e.*, there is a conspicuous absence of the rather even spacing of the threads observed in Figs. 22 and 23. Careful inspection of such cysts reveals various stages in pairing, conjugations, or synapsis of the chromatin threads (Figs. 25 and 26). In the same cyst one often finds various stages,—only one or two pairs and the other threads single as in Fig. 25, and all stages up to one in which all of the threads are in pairs. Occasionally some of the pairs in a nucleus show as much polarization as is indicated in Fig. 26, but in the same nucleus other pairs are differently distributed. There is never such complete polarization as is shown in some of the figures of Grégoire ('10), the Schreiners ('04), Agar ('11) and others. The occurrence of various stages of parasynapsis in the same cyst, the substantial agreement in size of the chromatin threads through the stages indicated in Figs. 23-26, and the spireme of double size in the next stage, preclude the possibility of interpreting the paired condition seen in Figs. 25 and 26 as a longitudinal split. Homologous chromosomes in some way come together, and gradually twist up into a tighter and tighter rope-like strand. A casual comparison of cysts in the stage shown in Fig. 27 with the earlier stages (Figs. 23 to 26), using low powers of the microscope, simply gives the impression that here we have a spireme, or sections of a spireme, twice as thick as in the previous stages, but study of such nuclei with Zeiss 1.5 mm. and oc. 12 reveals the double and twisted condition of the strands

FIG. 33. Anaphase showing segregation of homologous chromosomes and longitudinal splitting.

FIGS. 34-36. Second maturation mitosis showing dimorphism in number (18 and 19) and equational division.

in practically every nucleus, indicating that the paired threads are at no time so thoroughly fused as even apparently to lose their identity. This is further indicated by the earliest prophase stage (Fig. 28) where the paired strands begin to untwist. The following prophase stages consist of further untwisting and longitudinal contraction of the paired homologous chromosomes. In the synapsis stage (Figs. 25 and 26) it is impossible to tell whether threads of equal length form the pairs, but in the prophase pairs this is perfectly evident (Figs. 29 and 30). The untwisting and contraction frequently proceed at different rates in different pairs in the same nucleus and in different nuclei of the same cyst, so that one can easily compare the various stages of the process and be perfectly sure that the untwisting is continuous. There is no secondary fusion of paired threads such as frequently occurs in cases where a precocious longitudinal split appears in a telosynaptic bivalent and then closes up before the rings and crosses are formed (see *Blattilla germanica*, Stevens, '05). Fig. 30 shows various stages in the formation of the definitive chromosomes of the first maturation mitosis from the parasynaptic threads of a stage a little later than that of Fig. 28. The paired chromosomes untwist and contract simultaneously. Some remain united at one end (*b* and *d*) while in other cases union of a pair at one or both ends is a secondary phenomenon and may even occur after the spindle has formed (*a* and *c*). That there is much variation in the form and size of the 18 bivalents in metaphase is shown in Figs. 3, 7, 8, 9, 30, 31, 32 and 33. The most frequent forms are rings, E's and crosses, though one or more pairs of straight rods may be found in nearly every spindle. Most of the chromosomes are attached to the spindle fibers at or near the middle of each univalent member of the pair, so that the separated chromosomes pass to the poles of the spindle in the form of V's (Figs. 31 to 33). In the case of the double rods the fibers are attached at the ends. Many of the chromosomes are partly or wholly split longitudinally in the anaphase (Fig. 33). There are of course two kinds of second spermatocyte equatorial plates containing 18 and 19 chromosomes respectively (Figs. 34 and 35), X appearing in the form of a large V (Fig. 35) in one half of the cells. Division of the chromosomes is here longitudinal as seen in Fig. 36.

In *Ceuthophilus* the first maturation mitosis is therefore a segregating division of the previously paired and united homologous univalent chromosomes, while the second mitosis is as clearly an equational division of all of the univalent chromosomes including *X*.

DISCUSSION.

In an earlier study ('05) of the spermatogenesis of two other species of Orthoptera, *Blattella* (*Blatta*) *germanica* and *Stenopelmatus* (sp.?), I found what seemed to be good evidence of telosynapsis ('05, Pl. II., Figs. 55, 56, 58, 59, 62, 63, 64, and Pl. III., Figs. 108 to 115). That material I have reviewed and compared with the *Ceuthophilus* preparations, and I find no such evidence of parasynapsis in either of them. Naturally I expected to find telosynapsis in *Ceuthophilus*, and was surprised on working backward from the maturation mitoses to find no evidence of telosynapsis outside of the late prophases, and abundant evidence of parasynapsis in the young spermatocytes at a stage where synizesis is frequently found in other material.

A recent review of the literature on conjugation of chromosomes has only strengthened my previous conviction, based on my own experience with the spermatogenesis of a variety of forms, that the phenomenon is one which varies greatly in different groups of organisms, and even in different species of the same genus, or different sexes of the same species (*Sagitta*, Stevens '03, '05; *Bufo*, King '07, '08). Indeed I should not be surprised if the range of variation should prove to extend from (*a*) cases where there is nothing that could be called conjugation, but merely such a pairing, without contact even, as will secure segregation of homologous maternal and paternal chromosomes to different daughter cells, through (*b*) an intermediate condition of telosynapsis and less intimate parasynapsis, to (*c*) cases where homologous chromosomes are so completely fused in parasynapsis that it is impossible to tell whether the resulting chromosomes which are segregated in mitosis are identical with those that went into synapsis or not; and the variation may extend to cases which may give further support to Janssens' chiasma theory ('09) or to Morgan's modification of it ('11) in which homologous chromosomes are supposed to be twisted tightly together in parasynapsis and split across the twists in preparation for mitosis, giving daughter chromsomes which contain both maternal and paternal chromatin.

In *Ceuthophilus* the parasynapsis stage of Fig. 27 is intimate enough and long enough to favor the supposition that it is a true conjugation involving exchange of material particles or of chemical substances (genes), but there is no evidence of any splitting of Morgan's chiasma type. All of the evidence indicates that homologous paternal and maternal chromosomes twist together in parasynapsis and untwist in the prophase of the first maturation mitosis. In the flies and mosquitoes (Stevens, '08, '10, '11) we have examples of even more pronounced parasynapsis than in *Ceuthophilus*, but so far as I have seen, the indications are that the chromosome pairs twist up in synapsis and untwist in prophase much as in *Ceuthophilus; i. e.*, an opportunity for interchange of genes between homologous maternal and paternal chromosomes is furnished by the observed phenomena of parasynapsis in these forms, but no evidence of such a chiasma type of splitting after synapsis as is suggested by Morgan ('11) to account for the results of his breeding experiments with *Drosophila*. Such an exchange of parts of chromosomes as that described by Janssens ('09) might of course occur without being detected, at almost any point in the process of twisting or untwisting of the pairs, since the time element is not determinable in fixed preparations.

Moreover, it seems to me that, in view of the great range of variation in the phenomena of conjugation and segregation of the chromosome in the maturation of germ cells, cytological evidence from one form cannot safely be taken to serve as the basis of a theory or hypothesis to account for the experimental results on another form, but cytological and experimental work on the same form must go hand in hand, in order that any safe conclusions may be drawn from the results.

There seems to be no question but that synapsis, or conjugation of the chromosomes is the most difficult phenomenon connected with the maturation of the germ cells, to interpret correctly, and doubtless earlier parasynaptic stages have been overlooked in some cases where telosynapsis alone has been described in connection with the mitotic stages of maturation, but it seems to me quite unlikely that synapsis in all organisms follows one method; and, moreover, I believe that the variations in method of synapsis and intimacy of union of homologous chromosomes in different forms will be found to be directly connected with variations in methods of inheritance of unit characters, especially in relation to interchange or lack of interchange of maternal and paternal genes. If this is true we should expect to find more cases of complete coupling of unit characters where telosynapsis or no real synapsis occurs. If parasynapsis is an adaptation to secure interchange of genes, we should expect to find cases of telosynapsis followed by parasynapsis, as indicated, but not certainly demonstrated in the guinea-pig (Stevens, '11, Figs. 9, 10, 11). In my studies on spermatogenesis of the Coleoptera ('05, '06, '08, '09) I found evidence of telosynapsis in several cases and no evidence of parasynapsis, but this was only an incidental matter at the time, and of interest merely in relation to the segregation of whole chromosomes in the maturation mitoses. It is my intention to reëxamine all of my Coleoptera and Diptera material with reference to the questions whether parasynapsis occurs in the Coleoptera, and whether the Diptera show any evidence of Janssens' chiasma types of synapsis.

Bryn Mawr College,
January 1, 1912.

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FURTHER OBSERVATIONS ON SUPERNUMERARY CHROMOSOMES, AND SEX RATIOS IN DIABROTICA SOROR.

N. M. STEVENS.

SUPERNUMERARY CHROMOSOMES.

In the summer of 1910 while I was enjoying the privileges and hospitality of the Marine Biological Laboratory at La Jolla, California, I took advantage of the opportunity to study the male germ cells of *Diabrotica soror* from a new locality. Having previously ('08) found supernumerary chromosomes varying in number from one to five in about 50 per cent. of the male individuals of *Diabrotica soror* at Mountain View, California, and *Diabrotica 12-punctata* at Bryn Mawr, Pa., I was interested to see whether supernumeraries would be found in the same proportion in a third locality.

Testes from a hundred individuals were studied in aceto-carmine preparations. The greater part of the material was collected in a corn-field in the open country between La Jolla and the new laboratory which is two miles north of the town. A few were obtained from a rose-garden in La Jolla and one lot of 68 males and females was collected for me by Miss Myrtle Johnson on corn in a garden at National City, just south of San Diego. Individual records were kept for each lot, but the conditions with respect to number of supernumeraries proved to be about the same for the three collecting grounds.

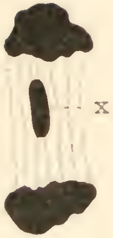
To my surprise I found supernumeraries scarce. In the first 25 males examined, 21 had no supernumeraries and 4 one; while out of the first 25 examined the same summer at Mountain View 15 had no supernumerary, 7 one and 3 two; and in the first 25 at Mountain View in 1909, there were 13 with no supernumerary, 9 with one, 2 with two and 1 with three. In the La Jolla material the 89th individual was reached before a case of two supernumeraries was met with, and in the first 100 males 79 had no supernumerary, 20 one, and 1 two. The following table shows the per cent. of supernumeraries in the two species different years and in different localities.

Number of Supernumeraries.	0	1	2	3	4	5
D. sor., Mt. V., '07, June 23–Aug. 7.	51	35	11	2	1	
D. 12–p., B-M., '07, Oct. 3–9.	48	33	15	3	1	
D. s., Mt. V., '09, July 10–Aug. 12. .	43	44	10	3		
D. s., Mt. V., '09, Aug. 21–Sept. 15.	46	38	10	4		2
D. s., Mt. V., '10, July 28–Sept. 1. . .	52	29	16	3		
D. s., La J., '10, June 17–July 4. . . .	79	20	1			

As I had never seen any signs of degeneration of the supernumeraries, the natural interpretation of their infrequency at La Jolla would seem to be either that they had originated here more recently, or that they had originally appeared in fewer individuals in this locality.

The behavior of supernumeraries in all cases where they have been shown to occur at once classes them with the heterochromosomes, and in *Metapodius* Wilson ('09) has shown that they have probably originated in an irregular second maturation mitosis in which both idiochromosomes went to one pole of the spindle instead of separating. He therefore regards the supernumeraries in *Metapodius* as duplicates of the smaller idiochromosome. In 1908 I suggested that there might be two varieties of *Diabrotica soror* and also of *D. 12-punctata*, one having only the odd heterochromosome and the other an unequal pair, and that hybridization might have given rise to the supernumeraries with their peculiar behavior, dividing sometimes in one sometimes in the other maturation mitosis. I have, however, been able to find no evidence in favor of this view. In 1910 I studied carefully the testes of many individuals where no supernumeraries were present, seeking some clue to the origin of these chromosomes.

As a rule the odd chromosome X appears near one pole of the spindle in the metaphase of the first maturation mitosis, but I had always noticed that occasionally X is in or near the equatorial plate, and in some individuals this is quite common. At La Jolla I found two spindles in which X was between the daughter plates in the anaphase, and stretched out lengthwise (Figs. 1 and 2). In one of these cases (Fig. 2) X was split so



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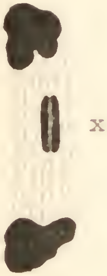

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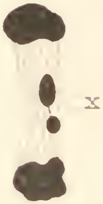

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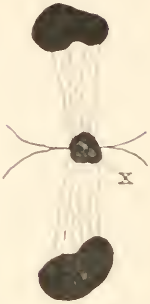

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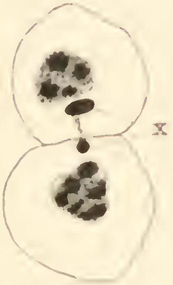

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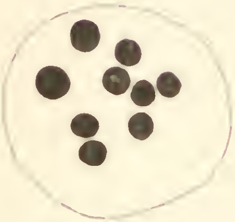

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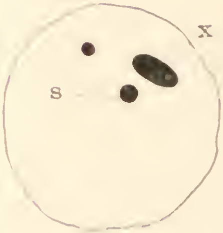

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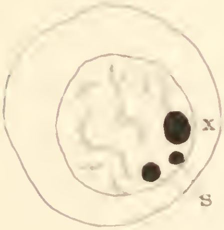

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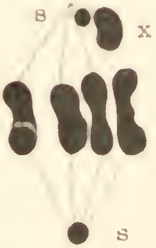

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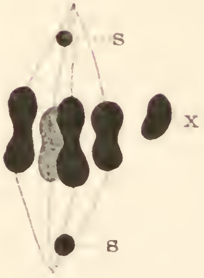

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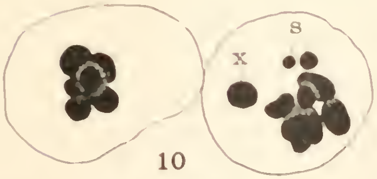

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FIGS. 1–5. Anaphases of first maturation mitosis, showing abnormal position and transverse division of *X*. (Mag. 1,500 for all figures.)

FIGS. 6*a* and *b*. Metaphase of first maturation mitosis, showing two supernumeraries (*s*) unequal in size.

FIGS. 7–10. Other stages from same testis showing behavior of *X*, and the two supernumeraries.

that it was certain that it was in a position such that it might divide transversely, but I was not able to find any cases of actual transverse division of X. Later at Mountain View I did find two anaphases where X appeared to have divided transversely and unequally (Figs. 3 and 5) and one in which X was caught in the cell plate between the daughter cells (Fig. 4). Now the supernumeraries are usually very uniform in size and certainly less than one half the sixe of X. I have one individual noted as having an unusually large supernumerary, about one-half as large as X, and a few cases where an unusually small one occurs. One of the latter cases is shown in '08, Pl. III., Figs. 76 to 78. From the evidence now at hand I should infer that the probable origin of the supernumeraries in the *Diabroticas* has been an occasional transverse division of X followed by a longitudinal division of the two parts. Evidently the transverse division has usually been an equal one, but that it may be unequal is shown by Figs. 3 and 5, and the rather rare occurrence of unusually large and unusually small supernumeraries. Figs. 6 to 10 are from a male captured at Mountain View, July 29, 1910. Here we have a large and a small supernumerary in the same individual. In the metaphase (6*a* and 6*b*) X and the two supernumeraries were all near one pole of the spindle, while in Figs. 8 and 9 the supernumeraries are at opposite poles and in Fig. 9, X is near the equatorial plate. In Fig. 10, X and both supernumeraries have gone undivided to one second spermatocyte. No cases of the division of either supernumerary in the first maturation mitosis were found in this individual.

In *Metapodius* Wilson found no somatic variations corresponding to the variation in the number of supernumeraries. In fact the insects with X alone, X and Y, or X, Y and 1 to 6 supernumeraries are described as indistinguishable. These species of *Diabrotica* are very variable in size, and in regard to size and fusion of the 12 black spots on the elytra, but as I showed in 1908 there is no significant correlation between these somatic variations and the presence or absence or number of supernumeraries ('08, Tables I. and II., and p. 465, text). In *Metapodius* the indications are that the chromosome Y is of no hereditary value. and the supernumeraries, as duplicates of Y would not be expected to affect the somatic characteristics of the insects. If, however, the supernumeraries of *Diabrotica* come originally from different regions of X, there would seem to be no reason why they should not bear functional genes for sex and other characters. The male always contains X so far as my experience goes (over 700 males), but one would suppose, if the supernumeraries are functional in heredity, that one X and a supernumerary might frequently determine the development of a female, and if so there should be males without X, but with a supernumerary in its place. It may, of course, be true that the abnormal division of X producing supernumeraries in itself indicates a degenerate or non-functional condition of that particular X chromosome, and therefore of its progeny—the resulting supernumeraries. This would fall in line with Schleip's ('11) suggestion in regard to the rejected X chromosome in the spermatogenesis of the hermaphrodite generation of *Angiostomum nigrovenosum*, that it had already become non-functional at an earlier stage, whence its later behavior. It is exceedingly desirable that the female sex cells of these *Diabroticas* should be studied, but I have not been able to get any favorable mitoses in the adults, or to secure larvæ or pupæ from the soil or roots of plants on which they live. Several attempts to breed them have given no results.

The testes of 12 males each having one supernumerary were studied from the point of view of the division of the supernumeraries in the first maturation mitosis. All anaphases and metaphases in each preparation were examined and all cases where it was possible to determine the position and behavior of the supernumerary recorded. In the metaphase the supernumerary was in the equatorial plate in 54.1 per cent. of 901 cases and out of the plate—nearer one pole of the spindle—in 45.9 per cent. Apparently the supernumeraries, when they divide, do so later than the bivalent chromosomes, so all anaphases were examined on this point. In 55.6 per cent. of the anaphases found in the 12 testes, the supernumerary was between the daughter plates, and in 44 per cent. it was dividing or divided. Here the 56.6 per cent. corresponds closely with the 54.1 per cent. in the equatorial plates in the metaphase, and the 44.4 per cent. outside of the daughter plates in anaphases comes very near the 45.9 per cent. out of the equatorial plates in metaphase. The division of supernumeraries or their failure to divide in the first maturation mitosis seems to be a matter of chance, depending on their position in the spindle in the prophase and on the attachment of spindle fibers from one or from both poles of the spindle. In Fig. 11 both supernumeraries are connected by fibers with both poles, in Fig. 12 the *s*-chromosome is connected with both poles and is about to divide, and in Fig. 13 one *s*-chromosome is connected with both poles and will later divide, while the other will go undivided to the upper pole of the spindle and therefore to one second spermatocyte. The behavior of the other chromosomes indicates a more or less definite attachment point for the spindle fibers, near the middle of the chromosome in both spermatogonial and spermatocyte mitosis (Figs. 11 and 13). The supernumeraries seem to be able to make connections with both poles in most cases if they are in or near the equatorial plate in late prophase stages.

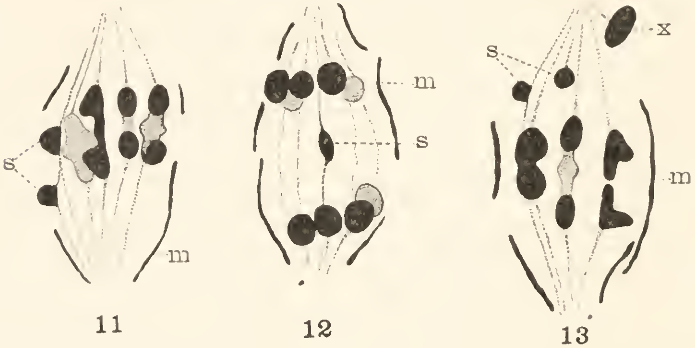

FIG. 11. Spindle showing two supernumeraries (*s*), each attached to spindle fibers from both poles. Mag. 2,000.

FIG. 12. Anaphase showing supernumerary (*s*) about to divide.

FIG. 13. Metaphase showing *X*, a supernumerary (*s*) attached to one spindle fiber, and another (*s*) attached to two. *m* = mitochondria.

SEX RATIOS.

The sex ratios in *Diabrotica soror* and *Diabrotica 12-punctata* have shown very peculiar variations. In studying the male germ cells of *D. soror* in 1907 I made no note of the number of females found in random collections, but in dissecting *D. 12-*

punctata in October, 1907, I found more than two males to one female,—in one lot 58 males to 25 females. In 1909 the number of males and females was noted for each lot dissected. Between July 10 and August 12, 107 males and 102 females were counted in random collections from two neighboring gardens, but it was noticed that the ratios in the two gardens were quite different. In garden *A* there were 58 males to 26 females; in garden *B*, 49 males to 76 females. A second lot from garden *A* collected between August 21 and September 15 gave 101 males to 24 females. The percentage of females in garden *A*, first lot, was 30.9, second lot 19.2, average 23.9, and for garden *B* 60.8.

At La Jolla in 1910 the ratios ran more evenly.

	♂		♀
La Jolla, June 17 and 18	25	:	16
National City, June 22	34	:	34
La Jolla, June 28	14	:	19
La Jolla, July 1	14	:	25
La Jolla, July 4	20	:	27
	107	:	121

At Mountain View again the ratios were peculiar. Five random collections in Garden *A* gave 100 males to 20 females, and two other later collections 76 males to 18 females. Only a few were collected from garden *B* giving 12 males to 6 females. In 1911 mixed lots from both gardens gave more males than females, 61 : 47. These were recorded incidentally while fixing a lot of testes for sections. By referring to the table on page 232, it will be seen that the number of supernumeraries runs about the same for the first 100 in 1909, about one half of which came from each garden (*A* 51 and *B* 49), and for the second 100, all of which came from garden *A*. It therefore seems unlikely that the supernumeraries have anything to do with the difference in sex ratios in the two gardens. The soil in garden *A* is harder in summer—more adobe in it—and less thoroughly cultivated than *B*. Two possibilities are suggested in this connection: (*a*) The males may be more successful in pupating and escaping from the hard soil than the females or (*b*) few of either sex may emerge from the hard soil in garden *A*, and the males may be better flyers and so come in larger numbers from other neighboring gardens. The latter is regarded as more probable.

The Bryn Mawr *Diabroticas* of 1907 were all collected on a large clump of golden rod in a pasture that had not been cultivated for many years, and they may have come out of the ground in the immediate neighborhood or from more recently cultivated fields near by.

These erratic sex ratios are probably merely another example of the interference of external conditions in what would otherwise be an equality of sexes, or in other words a shifting of normally equal sex ratios, or partial exclusion of one sex by peculiarities in the environment. The collections were all random in the sense that all the individuals that could be found were collected each time.

Bryn Mawr College,
January 3, 1912.

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THE RELATION OF THE FIRST CLEAVAGE PLANE TO THE ENTRANCE POINT OF THE SPERM.*

ERNEST E. JUST.

During the summer of 1911 at the Marine Biological Laboratory under the direction of Professor Frank R. Lillie, I was engaged in the study of the eggs of *Nereis* of certain cytological problems the results of which will appear later. The question of the relation of the entrance-point of the sperm and the first cleavage plane occurred to me. A very pretty method made possible in a satisfactory fashion the determination of this relation the results of which this paper embodies. I here take this opportunity to express my thanks and sense of gratitude to Professor Lillie for his inspiring interest in the work of which this is a part.

Material and Methods.

The eggs of *Nereis* when shed are irregular in shape due to pressure while in the body of the female. They soon fill out in the sea water, measuring about 100 μ equatorially and somewhat less in a polar direction. There is, however, a great deal of individual size variation in the eggs of a given female The eggs are almost transparent, colored a pale green by numerous deutoplasm spherules distributed throughout the endoplasm; around the equator is an irregular double girdle of 14 to 22 oil drops (Fig. 1). In polar view the large germinal vesicle appears to be in the center of the egg. It is, however, slightly elongated in the polar direction. The polarity of the ovocyte is, therefore, expressed by the polar flattening already mentioned, the position of the oil drops, and the form of the nucleus.

As has been shown (Lillie, '11) there are not two membranes in the unfertilized egg of *Nereis*, but rather a single vitelline membrane external to the radially striated cortical layer ("zona radiata," Wilson) of the egg. The ovocyte remains thus with nucleus intact until inseminated or otherwise stimulated—as for instance, by squirting forcibly through a pipette.

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*All drawings, of living eggs, made with the aid of a camera lucida.

Two or three minutes after insemination, a jelly is rhythmically extruded from the cortical protoplasm. In ten minutes the germinal vesicle breaks down, development is initiated.

Males and females captured in the evening while swimming at the surface of Eel Pond were kept in separate dishes until morning when they were transferred to fresh clean sea-water. To get an abundance of eggs and of sperm for an experiment, it was merely necessary to cut open a female and a male. The cut animals were removed from the dishes at once; moreover, every other precaution was taken to avoid abnormalities superinduced through toxic influences, mechanical shock, etc. In several watch glasses of sea water in which India ink had been ground up eggs were put together with a minute quantity of sea water containing very few spermatozoa. The time of insemination was noted and the numbered dishes observed to the second cleavage. This method was varied somewhat as I shall later note.

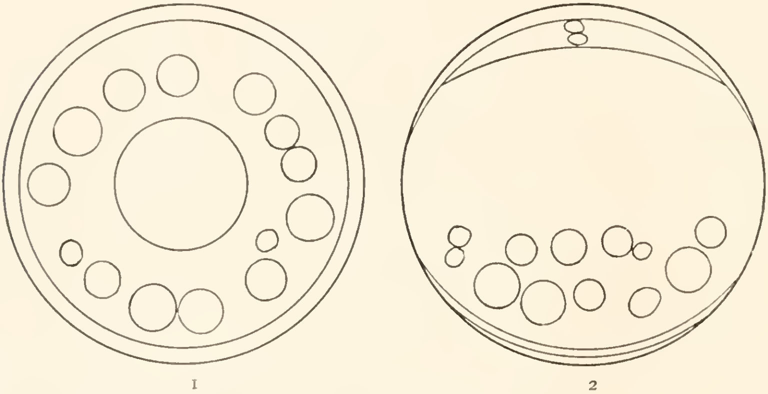


FIG. 1. Egg of *Nereis* at time of insemination; polar view.

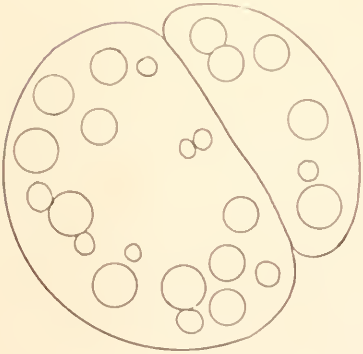
FIG. 2. Maturation stage; second membrane formed; oil drops at vegetative pole.

Ringed slides also were used; eggs placed on these in sea water and ink were inseminated. Sometimes a cover slip was placed on the eggs. Finally, for the later observations a very few eggs were placed on slides and the cover slips supported with glass rods.

Observations.

Outline of Development to First Cleavage.

Eggs in sea-water in which India ink has been previously ground up show clearly the formation of the jelly, the formation of the fertilization cone, and the entrance of the spermatozoön. A streak of ink points like a dagger or an exclamation point to the entrance cone above which on the membrane the spermatozoön is attached (Fig. 4). This "exclamation point" is an aid quickly to determine in a large number of eggs the relation of the sperm entrance-point. The spermatozoön enters the egg at any point. (So also Lillie, '11.)



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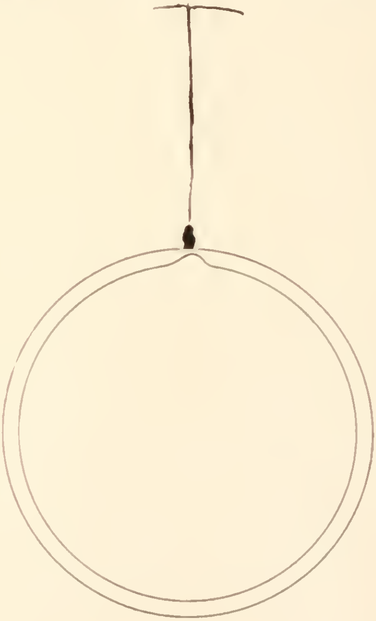

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Fig. 3. First cleavage.

Fig. 4. Cone and indicator formation, 15 minutes after insemination. Outer line marks boundary of jelly.

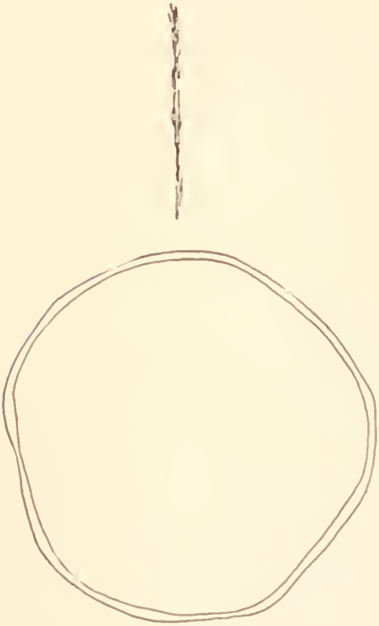
This ink "exclamation point," or "sperm indicator" as I shall call it, is a very interesting and striking formation worthy of detailed study. With me, however, the interest lay not so much in the formation of this indicator as in its availability to help answer the question: What is the relation of the sperm entrance point to the first cleavage plane? I here, therefore, give only as much of an outline of its formation and of the development of the egg to the time of first cleavage as will suffice to render intelligible the subsequent record of observations.

Almost at the moment the spermatozoön touches the egg membrane, the contents of the cortical layer begin to flow out as a viscid transparent substance of the same refractive index as water, leaving only radiating lines across the space (perivitelline space) between protoplasm and membrane which represent the walls of the emptied alveoli. This jelly in its flow carries the ink from the periphery of the egg so that between each egg and the surrounding ink is a clear space. This outflow of jelly may last for fifteen minutes. The jelly forms about the egg a layer everywhere continuous except along the tail of the sperm which thus forms a canal that increases in length as the jelly area widens.

Below the spermatozoön, the protoplasm of the egg begins to form a cone at thirteen to fifteen minutes after insemination which gradually increases in height until it reaches the membrane and then slowly retrogresses. With this retrogression, the membrane at this point sinks; in this depression lies the sperm. During this behavior, as the jelly area widens, the canal in the jelly in which the tail of the sperm lies fills in with particles of ink. This process is a gradual one, the indicator reaching its maximum of development fifteen to twenty minutes after insemination. The indicator, therefore, is formed along the tail of the sperm and points to the entrance-point of the sperm.

Twenty minutes after insemination, the spermatozoön may be seen attached to the membrane at the end of the indicator. The perivitelline space now becomes slight. The egg "assumes an amœboid appearance" (Wilson), changing its shape and becoming very irregular. The sperm cannot be seen readily (Fig. 5). About forty minutes after insemination the egg becomes spherical again. The sperm is easily visible on the membrane which is more widely separated from the protoplasm by the perivitelline space.

This condition is of short duration for the egg begins another series of changes. The membrane appears everywhere equidistant from the egg except at the point of sperm attachment where it is nearer the membrane. Then gradually to the right and left of the point of sperm attachment the perivitelline space becomes greater; the egg elongates along a line through the point of sperm attachment (Fig. 6). With the disappearance of the sperm head within the egg (about fifty minutes after insemination) this elongated appearance is lost (Fig. 7): the egg rounds out. The egg flattens at the animal pole (Fig. 8) and the polar bodies are given off from a clear apparently yolk-free region of the flattened pole (Fig. 2). Some little time later the first cleavage furrow appears and the egg is divided unequally (Fig. 3).



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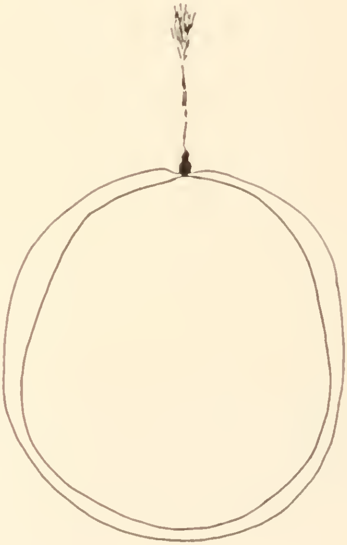

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FIG. 5. After retraction of cone; membrane close to the egg. Sperm apparently in the egg.

FIG. 6. Two minutes before sperm is engulfed.

The observations on the relation of this cleavage to the entrance-point of the sperm will be considered under three heads corresponding to the methods used.

Watch Glass Series.

A female was opened at 9:58, a male at 10:00. In five watch glasses of india ink ground up in sea-water eggs and sperm were mixed at intervals of two minutes. At 10:10 a few eggs were inseminated in the ink solution on an uncovered slide (no. 6). About two minutes after an insemination the jelly began to form; in fifteen minutes the sperm indicator was well developed. Eggs inseminated at 10:15 in a watch glass (no. 7) were washed at 10:30: that is, when the indicator had reached its maximum of development.

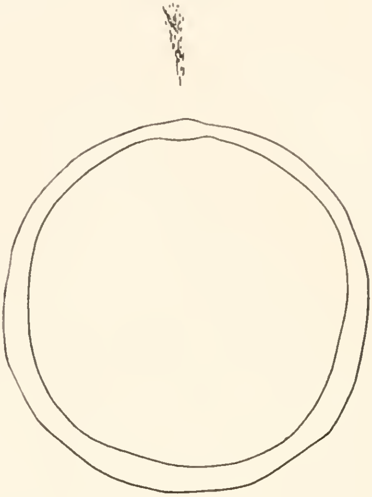

FIG. 7. Just after disappearance of sperm within the egg.

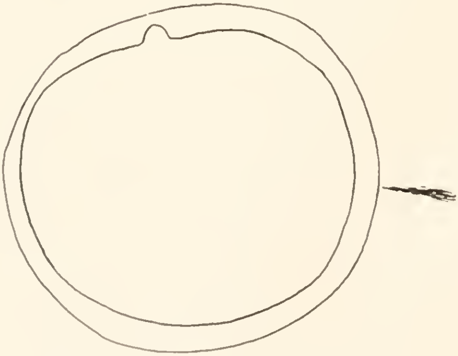

FIG. 8. First polar body forming.

The dishes (nos. 1 to 5) and the slide (no. 6) were examined as the first cleavage furrow appeared. In 95 per cent. of the eggs the first cleavage plane passed through the point of sperm entrance (Fig. 9). Dish no. 7 showed, on the other hand, that in only 50 per cent. of the eggs the first cleavage furrow passed through the point of sperm entrance.

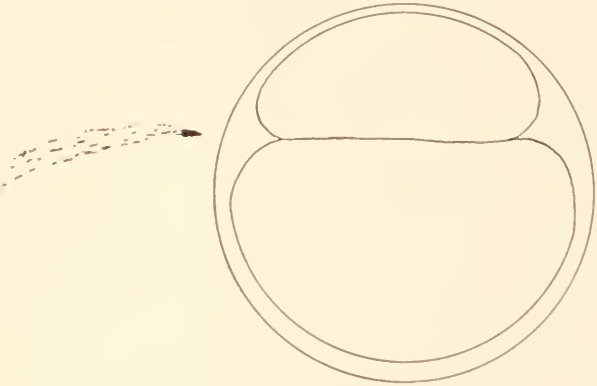

Fig. 9. First cleavage.

At 2:45 p.m. of the same day, eggs were inseminated in watch glass no. 8. Examination revealed that the first cleavage plane passed through the point of entrance in 80 per cent. of eggs. Eggs transferred from india ink and sea-water to clean fresh sea-water twenty to thirty minutes after insemination showed 60 per cent. of first cleavages through the point of entrance.

A summary of the results of Experiments 1 to 8 is as follows:

Nos. 1–5 inseminated in watch glass, not washed, showed first cleavage through entrance point, 95 per cent.

No. 6 inseminated on slide glass, not washed, showed first cleavage through entrance point, 95 per cent.

No. 7 inseminated in watch glass, washed, showed first cleavage through entrance point, 50 per cent.

No. 8 inseminated in watch glass, not washed, showed first cleavage through entrance point, 80 per cent.

No. 9 inseminated in watch glass, transferred to slide, showed first cleavage through entrance point, 60 per cent.

That the ink is not toxic to the eggs and, therefore, does not inhibit cleavage I was able to prove by inseminating at the same time two dishes of eggs, one with ink and one without; development in both went on at the same rate and in perfectly normal fashion. I concluded, therefore, that it was not necessary to wash the eggs. Also, I found later that the eggs were often too greatly crowded and that it was hard to make counts unless the eggs were in a single layer. A trial made with very few eggs unwashed in four watch glasses gave the following result (actual numbers are given):

First Cleavage Plane.

Number.	Through Entrance Point.	Not Through Entrance Point.
1	8	2
2	16	4
3	10	1
4	12	3

To what extent the eggs might rotate in the jelly was yet to be determined. It was absolutely necessary that the relation of the indicator and the sperm entrance-point remain constant; otherwise, the indicator would prove a very pretty but useless phenomenon. Could it be possible for two spermatozoa to reach the egg and the indicator to form along one sperm and not the other? How would such an egg cleave? These points were next to be determined.

I found, first, that the position of the indicator could be altered through tilting the watch glass, for the eggs would rotate in the jelly—especially when they lay on the side. I found later that the eggs are most liable to rotation after the sperm has disappeared. This might easily prove a serious source of error. Secondly, I demonstrated in several experiments that polyspermic eggs are not apt to cleave. (Professor Lillie has obtained the same results.) But with fairly dilute sperm and sea water, polyspermy, which merely cuts down the number of cleaving eggs, may be avoided.

In this connection it will be interesting to note the results obtained with old eggs and sperm. On July 30 eggs from a female captured in the evening of July 28 were used with fresh sperm—of a male captured in the evening of July 29. These eggs proved very susceptible to polyspermy. This proved true in other trials. These eggs if they segmented at all showed sixty per cent. of first cleavages through the entrance-point of the sperm. In general, eggs that have stood in sea water for some time after leaving the female, show a low per cent. of cleavages through the entrance-point. Five hours after leaving the female eggs fail to develop on insemination.

These results seem to indicate that the first cleavage tends to pass through the sperm entrance point—*i. e.*, through the point at the end of the indicator—if the eggs be fresh, undisturbed and fertilized with a single sperm. Why then do some first cleavages fail to pass through this point? During this time a number of experiments made by day and often at night immediately after the capture of the animals showed essentially the same proportions.

Ringed Slide Series.

It was stated above, it will be remembered, that the egg tends to lie with either pole uppermost. If, however, the eggs are not disturbed those that settle on the side will so remain. The eggs are accessible to sperm at any point if not under pressure as at no time in this study they were. The first cleavage always cuts through the animal pole near the polar bodies. Obviously then, the question of the relation of the first cleavage plane to the entrance-spot of the sperm cannot be settled by the cleavage of those eggs in which the spermatozoa enter either at the point below which the polar bodies are extruded or at a spot 180° from this point.

In the next trial with very few eggs on ringed slides, those eggs in which the sperm indicator pointed either to the polar bodies or to a point 180° from the polar bodies were not counted. This trial resulted as follows:

First Cleavage Plane.

Number.	Through Entrance Point.	Not Through Entrance Point.
1	14	4
2	6	4
3	20	9

Other experiments with ringed slides showed about the same proportions.

For fear that the ringed slides were toxic owing to the vaseline used they were abandoned and slides with cover slips supported by glass rods as well as the open watch glass were used throughout the next series of observations.

Slides with Glass Support for Cover Slip.

Four or five eggs on a slide were watched continuously through the first cleavage, the indicator used merely to point out quickly the point on the membrane where the sperm was attached. Very few sperm were used in these observations, obtained through diluting several times the water which contained them. These observations were repeatedly made at night and at different times during the day. Some of the eggs failed to show the indicator and to develop. In all that segmented, *the first cleavage plane passed either directly through the entrance-point of the sperm or a degree or so from it, with the indicator parallel to the cleavage furrow* (Fig. 10). It is possible, as stated above, to keep the spermatozoön in view after the amœboid stage until it disappears within the egg. The middle piece is left without. With the aid of the middle piece, the character of the membrane at the entrance point (Fig. 7), and the oil drops near, it is possible absolutely to hold in view the exact spot at which the sperm was engulfed.

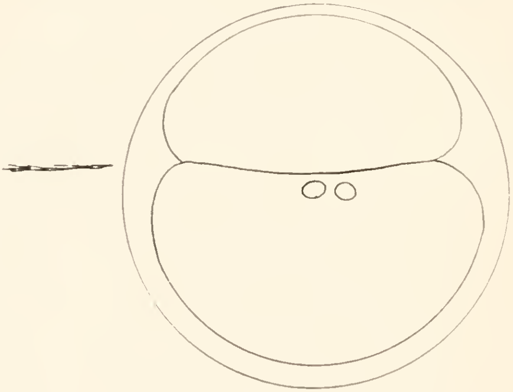

FIG. 10. First cleavage.

At intervals of two to three minutes, seven slides with very few eggs on each were prepared. Sperm was added and after a minute the eggs covered and every precaution taken to avoid disturbance. In the sixty eggs counted the first cleavage furrow passed through the sperm entrance-point in every case. In some cases the indicator appeared to be at right angles to the furrow but in all such it proved to be *above* the egg and ended in the cleavage plane (Fig. 11). This was Sunday, August 20. The laboratory was quiet, the temperature conditions favorable. The results of August 23, 24 and 27 are similar. Camera sketches were made of these eggs. Often I asked an investigator, who did not know the purport of the experiments, to make the sketches; the indicator without doubt was above the egg and pointed to the cleavage furrow.

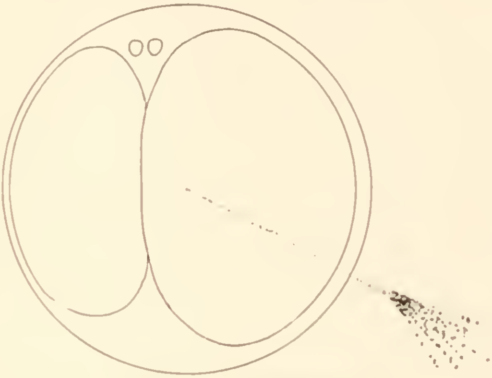

FIG. 11. First cleavage.

Discussion.

The first cleavage plane usually coincides with the median plane of the future animal in the frog's egg, according to Roux, Newport, Pflüger and Morgan. In the squid's egg also, according to Watasé, the first cleavage plane falls in with the median plane of the embryo. Agassiz and Whitman ('84) noted a like coincidence in the teleost egg; and Van Beneden and Julin, Castle ('96) and Conklin ('05) found that the first cleavage plane marks the long axis of the embryo in the ascidian egg.[1]

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[1] According to Harper, the sperm enters the pigeon's egg previous to the egg's entrance into the oviduct. He believes that the sperm must enter as soon as the disc is exposed through rupture of the follicular wall. In the pigeon's egg the sperm entrance is more or less localized. According to his figure, the first cleavage plane makes an angle of 45° with the long axis of the embryo. As we know from other researches, the long axis of the embryo is similarly placed in the egg.

But there are other eggs in which the future median plane does not fall in the plane of the first cleavage. In *Nereis* (Wilson, '92) the *second* cleavage plane, although it does not divide the animal into "equal halves," coincides with the long axis. So in *Crepidula*, the first cleavage plane is at right angles to the future median plane (Conklin, '97). In the newt (Jordan, '93) the case is the same. In *Chætopterus* (Lillie, '06) the axis of the first cleavage spindle lies in the longitudinal axis of the embryo.

There is a third group of eggs in which coincidence with any cleavage plane is wanting This is true of the egg of *Amia* (Whitman and Eycleshymer, '97), of the toadfish (Clapp, '91), and of certain amphibians (Jordan and Eycleshymer, '94), to name a few. And yet in most of these eggs the symmetry and the bilaterality of the cleavage may be sharply marked.

In the frog's egg the first cleavage plane usually and the median plane of the embryo always (*Rana fusca*) pass through the entrance point of the sperm (Roux, '85; Schulze, '99; Brachet).

In the egg of *Toxopneustes* (Wilson, '95) the first cleavage plane passes through the entrance-point of the sperm, "in the great majority of cases, at least." This plane of cleavage coincides with the transverse diameter of the embryo (Driesch).

In the ascidian egg, the belief of Castle ('96) is that the first cleavage plane cuts through the entrance-point of the sperm. Conklin ('05) says that there is no question but that the first cleavage plane is through the copulation path of the germ nuclei. And indeed his figures show very beautifully that this is actually the case.

If now we grant that in the egg of the frog and of *Toxopneustes* as in the egg of *Nereis* and of the ascidian the first cleavage plane is determined by the copulation-path, or the entrance-point, of the sperm we have this interesting conclusion: The first cleavage plane in eggs whose cleavages have different values and different relations to the future long axes of the embryos is determined by the entrance of the sperm. While the sperm entrance determines the first cleavage, the first cleavage does not in all of these forms coincide with the median plane of the future animal.

Since in the egg of *Nereis* the sperm may enter at any point and since the first cleavage plane passes through this point, the structure of the ovocyte of *Nereis at the time of insemination* must be the same in all meridians. This, I believe, has an important bearing on theories of germinal areas in the cytoplasm, of prelocalization, and of precocious segregation. The determination of bilaterality follows fertilization.

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PALMEN'S ORGAN AND ITS FUNCTION IN NYMPHS OF THE EPHEMERIDÆ, HEPTAGENIA INTERPUNCTATA (SAY) AND ECDYURUS MACULIPENNIS (WALSH).

J. E. WODSEDALEK.

INTRODUCTORY REMARKS.

Our knowledge concerning the tracheal system in the Ephemeridæ dates back to the time of Swammerdamm (1752), but the existence of this interesting modification, Palmen's organ, found only in the tracheal system of this group of insects, was not known until comparatively recent times. Swammerdamm in his "Bibel der Natur" gives a large figure (Plate XIV.), showing in some detail the internal anatomy of a may-fly nymph, but the Palmen's organ and even the four tracheal tubes directly leading to it, if present in that species, apparently escaped his observation. This omission was no doubt due to an imperfect dissection; for, upon closely observing his representation of the air tubes in the head of the nymph he figures, one can detect a single projection leading from the main tracheal tube on the left, which corresponds somewhat to one of the four tubes normally leading to this organ; the other three tubes and the organ itself were doubtless destroyed in his preparation, and hence not represented in his figure.

The presence of this chitinous structure was first noted by Dr. J. A. Palmen (1877), after whom the organ is named, and in his work he says: "Die vier im Scheitel zusammenstossenden Aeste bilden in ihrem Kreuzpunkt einen rundlichen, aus concentrischen Chitinschichten bestehenden Körper, dessen Bedeutung ich nicht kenne." On Plate I. (Fig. 7) he gives a figure of the head and thorax of the nymph of *Cloëon dipterum* L., showing the location of this organ in its relation to the four tracheal tubes of the head, without making any attempt to describe it. He makes the statement that the tracheal system is essentially the same in the twenty-three species which he examined. It is not entirely safe, however, to infer from this that the prominence of Palmen's organ is essentially similar in these various species.

The species upon which the present study is based are *Heptagenia interpunctata* and *Ecdyurus maculipennis*. These two forms are very closely allied, not only in matters concerning this organ, but also in their natural habits and general behavior, and the present paper will concern itself with nymphs of *H. interpunctata*, unless otherwise specified.

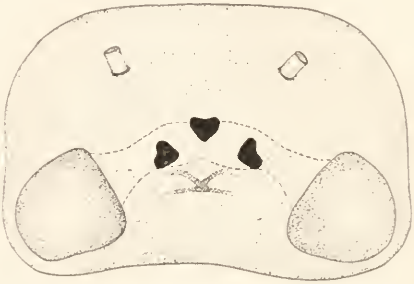

FIG. A. Head of *H. interpunctata* nymph. Basal joint of antennæ only drawn. The brain is drawn, dotted, under the three ocelli just posterior to which is shown the Palmen's organ and the four tracheal tubes leading into it.

Fig. A shows the relative position of the organs in the head of a nymph. Palmen's organ together with the tracheal tubes leading to it can be readily seen through the chitinous covering, especially in the newly moulted specimens, by placing them under a binocular. It is symmetrically located between the two large compound eyes and a little posterior to the brain. Fig. 1 shows the organ in its relation to the entire tracheal system of the head. It has been the fortune of the writer to be able to make a perfect dissection of the system, the first time merely through an accident. Upon placing a specimen which had been dead for some time under a binocular almost the entire tracheal system of the insect became visible through the transparent chitinous covering. The muscles and all the other soft tissues had sufficiently decomposed to form a sort of liquid mass. The thin hypodermal walls surrounding the air tubes too had disintegrated, and practically all that remained in good condition was the exoskeleton and its internal continuation, the tracheal system. The location and arrangement of the more important parts of the system were carefully noted and a diagram indicating the relative position of the main tubes was sketched. The external covering was carefully broken between the pro- and mesothorax and a gentle pull on the anterior edge of the head removed it, fully exposing the air tubes which remained in position. The macerated mass was carefully washed off and the tracheal system being completely filled with air presented the most beautiful silvery effect against a dark back-ground. Even the very finest branches remained, but no attempt was made to include them in the figure.

Description of Palmen's Organ.

Gross (1903) attempts to describe the organ in considerable detail; this description and his ideas in general are not fully corroborated by the results of my studies. He says in part: "Reconstruiren wir jetzt aus den besprochenen Schnittbildern das ganze Organ, so erhalten wir folgendes Gesammtbild. Ein Körper, der im Längsschnitt kurz elliptisch, im Querschnitt ungerfähr kreisförmig ist, setzt sich aus 14 concentrischen, aus zartem Chitin bestehenden Schalen Zusammen, die an ihrer Innenfläche mit feinen Härchen dicht besetzt sind. Das Ellipsoid ist aber kein vollkommen geschlossenes. Vielmehr ist es von vier Seiten her [sehr] tief ausgehöhlt. Das ganze ist in den Kreuzungspunkt von 4 im Scheitel des Hinterkopfs zusammentreffenden Tracheenästen eingeschaltet, und zwar so, dass die Luft zwischen den Schalen frei circuliren kann, wenn auch durch die grosse Zahl der Härchen einigermaassen behindert. Ganz ähnlich gestaltet wie bei *Ephemera vulgata* L. fand ich das Palmen'sche Organ noch bei *Baëtis rhodani* Pict., *Heptagenia sulphurea* Müll., ferner bei den Larven einer *Caënis* sp. und einer *Chirotonetes* sp. Einige geringe Abweichungen in der Gestalt bei *Baëtis rhodani* konnte ich nicht hinreichend genau feststellen, un sie hier zu besprechen, da ich mir nicht genügend Material beschaffen konnte."

The Palmen's organ in both *H. interpunctata* and *E. maculipennis*, is not composed of concentric shells nor are there any hairs present on the inner surface of the scales which Gross describes and pictures in allied species. Well prepared slides of cross sections show that the organ is not perforated with air passages but is a continuous mass of chitin in which the differentiations are due mainly to variation in density of color. Sections of adult specimens were also made and carefully examined, but no difference in the structure of the organ could be detected. Dr. Gross has no doubt mistaken the clearer areas or concentric layers for air passages and the darker layers for separate solid areas forming the scales from which the hairs protrude.

Fig. 4 shows the external dorsal appearance of the organ and its relation to the four tracheal tubes, the entire structure being enveloped by the hypodermal layer; Fig. 5 is a horizontal section of the same. The description of the organ can be best understood by studying it in connection with its development and growth. It is a well understood fact that the tracheal system in insects is formed by the invagination of the ectodermal layer. As to the origin of Palmen's organ I am not at all certain for embryonic material has thus far in this study not been available. The appearance of the structure of the central portion of the organ suggests that, during the process of the development of the tracheal system, the four large tubes leading to the organ (Fig. 1) come together at a common point; here the blunt ends of the invaginated portions, the tracheæ, surrounded by the hypodermis, fuse and secrete this common center. From the various cross sections of which Fig. 8 is typical, it can be inferred that the two posterior tubes come together first and that a portion of the center is secreted before it is met by the two anterior tubes.

In the many sections of *H. interpunctata* and *E. maculipennis*, which were examined, the center of the organ does not show any ring-like structure, but is an irregular mass which is apparently secreted before the larva casts the first lining of its tracheal system. At the time of this first ecdysis which is accompanied by the shedding of the inner lining of the air tubes, this central mass is larger than the openings in any of the four tubes and hence the impossibility of its being cast out of the body. Shortly after the casting of the inner lining of the tracheæ, the hypodermal cells surrounding the tubes undoubtedly begin to secrete the new chitinous wall. The hypodermal layer surrounding the central mass, the beginning of the Palmen's organ, is continuous with the layer surrounding the air tubes and apparently begins active secretion at about the same time. The different conspicuous rings which are shown (Figs. 5–9) are sections through the concentric layers of the organ and are directly correlated with the various moults. Further evidence of this correlation is obvious from the fact that the number of rings is directly in proportion to the size of the insects themselves. An examination of the sections figured shows that the hypodermal cells surrounding the organ are much larger than those enclosing the tracheæ, and hence, the greater the secretion of these larger cells; from this results the greater thickness of the chitinous layers of the organ as compared with that of their continuations, the walls of the tracheal tubes. Coincident with this increase of volume of the organ, the cells surrounding it must necessarily multiply as they are pushed outward. Thus, by means of successive periodic secretions the Palmen's organ is built up; the old layers of the organ are not cast off as are the walls of the tracheal system, with which they are continuous.

Gross in commenting on the function of Palmen's organ says: "Ich glaube deshalb, dass für das räthselhafte Organ keine Erklärung gefunden werden kann ohne Berücksichtigung des Nerven. Nehmen wir aber an, dass dieser wirklich zu dem Organ gehört, so kann dieses nichts anderes sein als ein Sinnesorgan. Da es aber, wenn auch ziemlich direct unter der Hypodermis—von dieser nur durch wenig Fettkörper getrennt—doch jeden Falls im Innern des Körpers der Thiere gelegen ist, kann es von allen uns von andern Thiergruppen bekannten Sinnesfunctionen nur denen eines Gleichgewichtssinnes dienen." Up to the present study no experimental work on the organ has been attempted with the view of obtaining evidence as regards its function. Gross also says: "Man könnte meinen, der Beweis für die Richtigkeit der von mir versuchten Deutung des Organs liesse sich vielleicht durch zweckmässig angestellte Versuche erbringen. Das erscheint mir aber ziemlich aussichtslos. Es wäre ja gewiss nicht unmöglich, das recht oberflächlich gelegene Organ zu zerstören, nachdem man vorher seine Lage so genau festgestellt hat, dass man sie schon von aussen am lebenden Thier angeben kann. Aber ich fürchte, dieses Experiment wird nicht viel helfen. Stellt sich nach dem operativen Eingriff irgend eine Aenderung der Flugweise ein, so kann diese auch durch die Verletzung an und für sich bewirkt sein. Wir wissen aus der experimentellen Gehirnphysiologie der Vertebraten zur Genüge, in welche schwere Irrthümer man geraten kann, wenn man die Verletzung oder Zerstörung eines Organs oder Organtheils als reinen Versuch betrachtet. Während man aber bei einem Wirbelthier wohl warten kann, bis die störenden Nebeneffecte des operativen Eingriffs verschwunden sind, so scheint mir das bein einer 'Eintagsfliege' kaum möglich zu sein. Selbst ein nicht zur Begaltung gelangtes Exemplar dürfte in der Gefangenschaft nur zu bald eingehen. Auch würden die Thiere wohl kaum den Hochzeitsflug aufnehmen, wenn man sie nicht in die ihnen zusagende, natürliche Umgebung und unter Artgenossen bringt. Thut man dies aber, so würden einem die Versuchsthiere gar zu leicht entschlüpfen, nachdem sie einmal aufgestiegen sind. Ebenso wenig Erfolg verspreche ich mir von dem Versuch, die Function des Organs durch Verkleben der in die Kopftracheen führenden Stigmen festzustellen."

Removal of the Organ.

Experimental work on the removal of the organ did, as Gross said, at first seem impossible. It is needless to say that the task was very tedious and at the outset far from encouraging, this was mainly due to the small size of the organ and its close proximity to the brain. At first the cauterizing method was used but without satisfactory results, then two very fine platinum needles which were attached to the two wires leading from a galvanic battery were employed. The apparatus was provided with a resistance box so that the voltage could be varied at will. In this method the end of one needle was turned into a small loop through which the sharp point of the other was inserted, thereby completing the current, heating the sharp point intended for the operation, and at the same time, greatly facilitating the necessary steady manipulation of the outfit. The hot point of the needle would be brought directly over the organ and then a rapid insertion and withdrawal of the point of contact followed. It was impossible at each attempt to destroy the organ owing to its natural instability. A few specimens from which the organ had been thus entirely removed, lived a sufficient length of time to enable studies of the behavior of the individuals, and of the regeneration of some of the destroyed parts.

Becoming more thoroughly familiar with the structure and exact position of the organ in its relation to the vital parts of the head, a more simple method was devised. By means of two very fine and sharp-pointed needles a small slit can be made through the chitin above the organ and then, inserting a needle at each side between the posterior and anterior tracheal tubes leading to the organ, it can with some practice, be entirely removed; this treatment apparently causes the insects but little pain. The four tracheal tubes were usually separated near the organ though sometimes they would break off near their juncture with the main longitudinal tracheæ. In special cases, for studies of regeneration of the organ, the four tubes were broken off at their immediate attachment to the organ or at various definite distances from it. This was possible by pressing the two points of the needles on either side of the place where the break was desired. Bleeding was very rare and usually the edges of the chitinous slit were brought so close together that the detection of the wound was rendered almost impossible.

After treatment by this method the activity of the nymphs when placed back into the water did not seem to be impaired by the operation, and the wounds healed over within a few days. By this method not only was the removal of the organ assured, but mortality was reduced to a minimum. In one set of experiments forty-nine out of fifty specimens operated on lived for more than two months after the operation. It might be said in this connection that no regeneration of the organ takes place. The ends of the broken tubes heal over within two or three weeks and with the exception of a few small air tubes which grow out from the blunt ends of the four tubes, during the same time, no further growth was observed in any of the specimens as long as four months after the organs had been removed. Fig. 3 is drawn from a nymph in which the tracheæ were broken off at their point of contact with Palmen's organ, they almost touched but no regeneration of the organ took place, nor was there a union formed between the different tracheæ Fig. 2 is of a specimen in which the tracheæ were broken at quite a distance from the organ; again, no growth beyond the covering over of the broken ends and the formation of a few small tubules took place.

COMPARISON OF THE BEHAVIOR OF NORMAL AND OPERATED SPECIMENS IN RELATION TO THE FUNCTION OF PALMEN'S ORGAN.

In my previous papers (Wodsedalek, '11 and '12), the behavior of *H. interpunctata* nymphs has been discussed in considerable detail, and hence only the more important phases of the behavior of this insect which directly concern this problem will be cited here. The nymphs are decidedly negative in their phototactic response in all gradations of light, varying from ordinary daylight to very intense electric illumination. Their thigmotactic propensity, or tendency to come in contact with and cling to objects, is especially pronounced. In their natural environment the nymphs are never seen swimming freely about in the water, even when observed in their favorite places in which they occur in great abundance. In their natural habitat they are always found clinging to the under surfaces of small rocks, and this same position is regularly assumed by all normal ones in the aquaria of the laboratory. When a stone, to which the specimens are attached is inverted in the water, the insects soon make for its under side, many of them doing this as the stone is being turned over. This is also true of normal specimens in the dark-room, and hence it is obvious that this tendency of the nymphs to cling to the lower surfaces of rocks, with their dorsal side downward, is not due entirely to their negative reaction to light. It is unquestionably due, in part, to a definite power of orientation independent of phototaxis.

Specimens from which the Palmen's organ was removed react to light in practically the same way as do the normal specimens. Their thigmotactic inclinations, too, do not seem to be impaired. However, when the insects are taken into a very shaded or a dark-room the difference in orientation becomes quite obvious. When a stone to which the insects are attached is inverted in the water, or when the specimens are dropped on a stone in the water in a dark-room they remain on the upper surface or on the sides of the rock for a considerably longer time than do the normal individuals By the removal of the organ the nymphs have no doubt lost, to some extent, their usual keen sense of orientation, for under such conditions they would be seen on the top, sides or any part of the rock for hours, days, and even weeks after the operation had been performed. The same was true of every lot experimented with. It was also noticed, with several lots of operated specimens, that the tendency to remain on almost any part of the stone was gradually diminished and that after several weeks and in some cases about two months there were comparatively few individuals to be seen on the upper surface, regardless of the fact that in some special experiments the stone would be inverted at every observation with the view of bringing more specimens to the upper surface with little disturbance. This growing partiality to the lower surface of the stone does not lessen the significance of their former behavior, for, from my studies on the power of the formation of associations in the nymphs of *H. interpunctata* (Wodsedalek, '12) it was found that they gradually formed several types of associations. The associations formed in these experiments were in connection with their thigmotactic inclinations, which were in great part responsible for the gradual decrease of the number seen on top, and the gradual diminishing of the time the various individuals required to retreat to the lower surface.

In another paper (Wodsedalek, '12) on the natural history and general behavior of these insects I have discussed their thigmotaxis in considerable detail. It was learned from a simple experiment that their thigmotactic propensities are best satisfied when their dorsal as well as their ventral surfaces are in contact with some object. "When several specimens are placed in an aquarium they mass together into clusters where they remain for hours, and if recently collected, even days. As soon as a rock or any other object is placed in the water the loose forms swim toward it, while considerable time often elapses before the masses are broken up. Two long bricks were placed one over the other in a basin of water and between them were placed small pebbles varying in size so that the space gradually varied in thickness from one end to the other. Then a large number of specimens were put in the water and after a short time it was found that nearly all of the specimens were attached to the lower surface of the upper brick with their dorsal sides downward, and a large majority of the specimens were in that part of the wedge-shaped space where their backs came in contact with the brick below." The operated specimens in their wandering about over the surface of the stone accidently came into such a place where their backs came in contact with the floor of the basin. This stimulus naturally appealed to their thigmotactic propensity and hence the greater tendency to remain on that portion of the rock. It seems only natural, therefore, that an association would be formed between this more satisfactory environment and the lower surface of the stone. It is not altogether improbable however, that such a habit had already been partially formed before the operation took place.

Further evidence for the fact that this thigmotaxis is largely responsible for the gradual disappearance of the insects from the upper surface, is apparent from the results obtained in some checking experiments. In those experiments the stone was suspended in the water so that the backs of the nymphs could not come in contact with other objects. The results were surprising and all remaining doubts as to the function of the Palmen's organ in the nymphs were resolved. As long as the experiment was continued the specimens remained quite evenly scattered over the entire surface of the suspended stone. A similar experiment was tried with the normal specimens, also in the dark chamber, and practically all of the specimens remained exclusively on the lower surface. It is only natural, then, to conclude that Palmen's organ has a great deal to do with the orientation of these insects. That this unusual behavior is not due to the shock the insects receive from the operation was proven by the fact that when other parts of the head and body were destroyed no comparable results in behavior took place.

Although the foregoing results are thoroughly convincing as to the function of the organ in these nymphs, further results of observations on behavior relative to the rôle of the organ may be cited. When the specimens are collected and dropped into a dish of water many of the individuals fall to the bottom with their ventral sides upward. This toppling over is even more obvious when the specimens are placed in a dish of water near a light. In their attempts to get away from the light and repeatedly clawing at the opposite end of the dish the specimens become exhausted and very frequently when the clawing movements cease the apparently lifeless individuals fall to the bottom, dorsal side downward. This period of rest corresponds somewhat to the death-feigning instinct of the insect. By vigorously stirring up the specimens or throwing them into water having a temperature to which the specimens are not accustomed, or into relatively strong chemical solutions of various sorts, as acids, salts, alcohol, etc., practically all of the specimens fall into this momentary, rather stiff, inactive state and slowly descend to the bottom of the dish. In so doing almost all of the specimens topple over and fall down head-first, ventral side up and on the average, at an angle of about 45 degrees. It might also be mentioned here that nymphs which are found dead in the aquaria lie almost invariably with their ventral side up. On the other hand, the turning over is under similar conditions far less frequent among the specimens from which the organ had been removed. If two groups of freshly killed specimens are taken, all of which have been cleaned and their appendages arranged, the one group normal in every way, the other having the Palmen's organ removed, we find by allowing them to descend through a deep jar of water that almost invariably the former topple over and settle on the bottom ventral side up, while the latter equally as frequently reach the bottom and remain there with their ventral side downward.

Concluding Remarks.

The results of the foregoing experiments show conclusively that the organ, as small as it is, plays a very important rôle in the behavior of the nymphs upon which these experiments were performed. This is doubtless due to the weight of the chitinous mass whose pressure seems, to a large extent, to control certain orientation of the insects. Gross (1903) gives a figure of the cross section of the head of a may-fly showing the position of Palmen's organ in relation to the other parts, and in his discussion says,—"Unter dem Palmén'schen Organ verläuft nämlich bei allen 5 von mir untersuchten Ephemeridenspecies ein starker, vom Gehirn kommender Nervenstrang. Seine Lagebeziehungen ergeben sich aus Fig. B, die einen Medianschnitt durch den Kopf einer Ephemera vulgata bei schwacher Vergrösserung darstellt. Der erwahnte Nerv (*np*) verlauft in der Medianlinie vom Gehirn (*g*) nach hinten unter dem Palmén'schen Organ (*p*) hindurch und heftet sich hinter ihm an der Körperwand an. In einem Theil seines Verläufs liegt er direct auf dem Nervus recurrens (*nr*) des unpaaren sympathischen Nervensystems."

Careful examination of many nymphs showed no evidence of the presence of the two large nerves which Gross speaks of as present in the imaginal species which he examined; this was also true of the adult specimens which I examined. It appears from his discussion of the subject and from his figure (page 98), that what he speaks of as nerves may possibly be the two muscles which play an important part in the movement of the head. The posterior attachment of these muscles to the exoskeleton evidently corresponds to the attachment of the large nerves he misrepresents. In my preparations very thin sections were made, but no signs of nerves extending directly from the brain to the organ were detected. Taking the structure and function of the organ into consideration we should not expect the presence of such nerves. A mass of rather loose tissue exists between the organ and the brain, and the two are loosely united by means of connective tissue. It is the writer's opinion that the chitinous organ being so loosely supported by the four tracheal tubes exerts a pressure on the surrounding tissues, whereby the disturbing stimulus reaches the central nervous system. The observations mentioned on the descent of nymphs in various conditions, through the water, particularly the death-feigning and the dead individuals, seems to indicate that the orientation is also, in part, a self-directing process, that is, by the presence of the organ the nymph is swerved into position—a matter of physical equilibrium.

Gross' theory that the organ functions only in the adult specimens seems quite untenable. Aside from the results of my experimental work arises another question. Why should this structure occur and persist in very small nymphs, and grow in relative proportion during the comparatively long nymphal stage of two, and in some cases three years, for the purpose of becoming functional only after the nymph metamorphoses into its short-lived adult stage, when all the other modifications which are of a direct advantage to the adults develop during the comparatively short time immediately preceding the transformation?

The extent of the functions of this organ in the adults thus far remains unknown. Miall (1895) in speaking of the Ephemeridæ gives the following quotation: "The recently emerged fly," says De Geer, "settles on trees, plants, walls, etc., near the water which harbored the larva. Here it fixes itself by the hooks of the feet, usually with the head downwards, and rests until the last or sub-imaginal moult is at hand." My observations of the behavior of adult may-flies are to some extent in accord with those of De Geer, however, no theory as to the probable function of the organ in the adults can be propounded, unless it can be supported by reliable results of experimental work. A large number of nearly full grown nymphs from which the Palmen's organ had been removed are now in the aquaria with the view of making a study of their behavior, when they emerge as adults in comparison with that of the normal individuals.

Among the twenty-three species in which Palmen (1877) noted the presence of this organ, there are several free swimming forms, and at this time, it is difficult to say just what part Palmen's organ plays in those forms during their life history as very little is known of their natural habits.

I am greatly indebted to Professor William S. Marshall for suggesting this problem, and also, for his help and earnest interest in the progress of the work.

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EXPLANATION OF FIGURES.

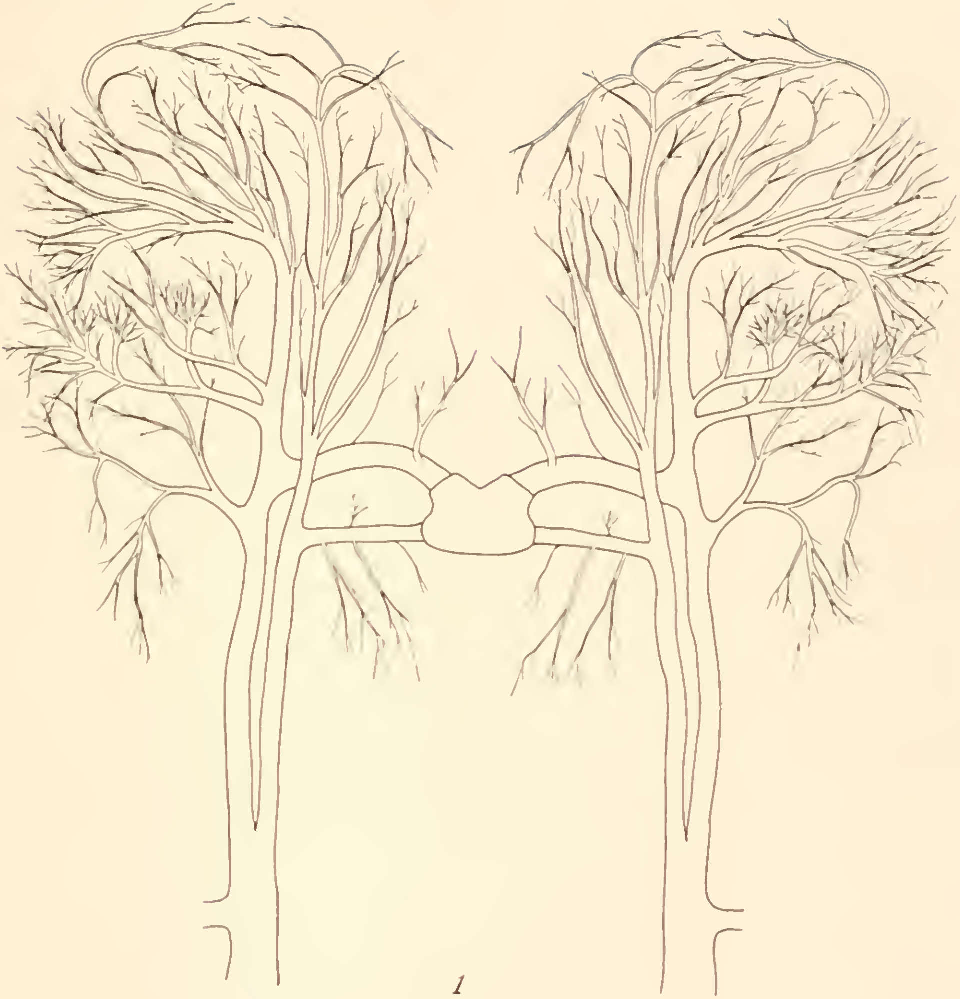
Plate I.

All drawings (except Figs. 2 and 3) made with a camera-lucida. ×240.

Fig. 1. Palmen's organ in its relation to the tracheal system in the head of the nymph *H. interpunctata*. × 60.

Fig. 2. Sketch drawn from a specimen which had the organ removed and the four tracheæ broken off near their juncture with the main longitudinal tubes.

Fig. 3. Sketch drawn from a nymph in which the tracheæ were severed at their point of contact with the organ.



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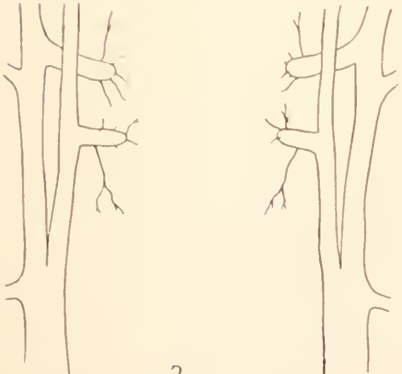

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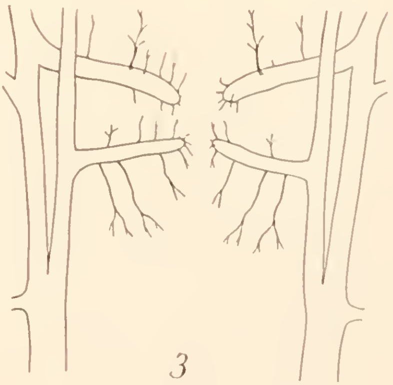

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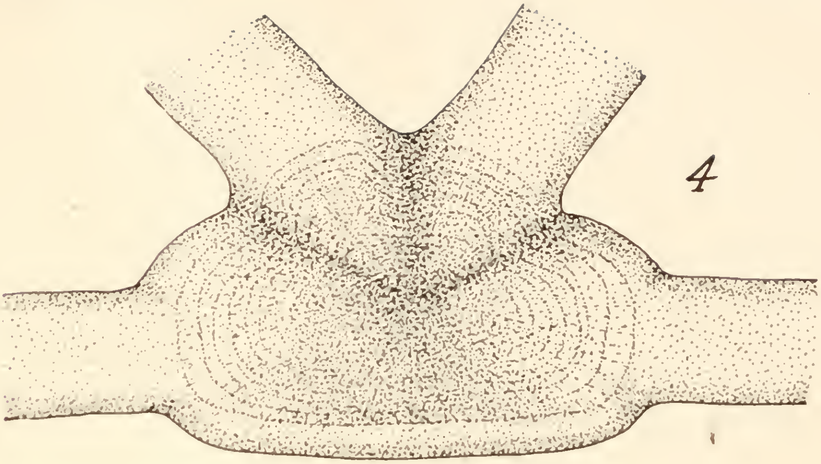
Plate II.

Fig. 4. Representation of the entire organ surrounded by hypodermis, as it appears in a mounted specimen. Circular bands can be seen, especially at the edges of the organ, owing to the fact that we look at the vertical portion of each deeply colored part. In this view the large light areas appear at the entrances of the tracheal tubes; this is due to the fact that we look through a comparatively thin portion of the chitin in those regions owing to the direct extension of the cavities of the tubes into the organ. The darker areas appear as such because of their thickness; each is a concentric mass around the organ and forms the partition between the cavities.

Fig. 5. A horizontal section almost directly through the center of the two posterior tubes and a little above the center of the two anterior ones. It is only natural, therefore, that the two posterior tracheæ should lead to the solid central mass. The entrances of the two anterior ones are not in the same plane with that of the posterior pair and therefore the innermost portion of their cavities are not represented in this section. The gradually increasing diameter of each cavity is understood when we recall the development of the organ and the tubes leading to it.

Fig. 6. A horizontal section through the ventral projection of the organ which is apparent in Figs. 7 and 8. The central part of this figure appears clear because the section was quite thin and the cut parallel with the light portion of one of the concentric layers.



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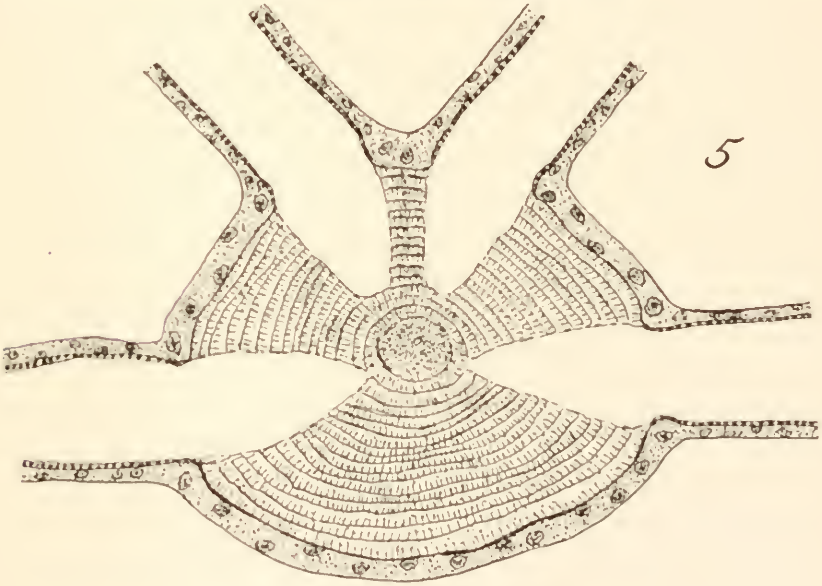

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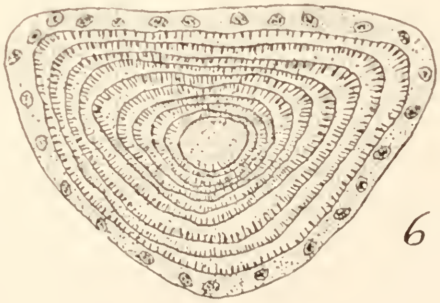

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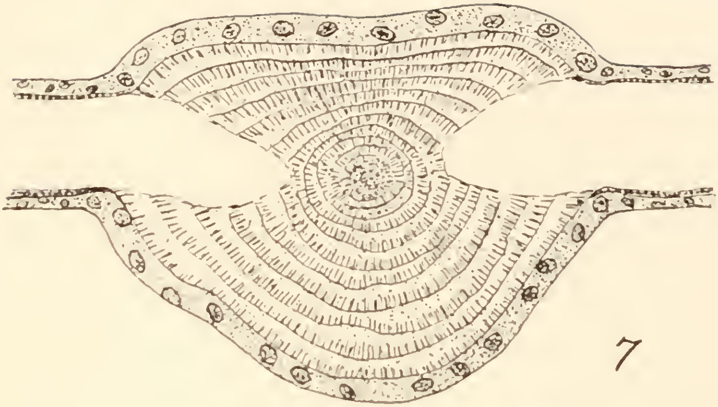
PLATE III.

FIG. 7. A transverse section cut near the center of the posterior pair of cavities.

FIG. 8. An oblique transverse section cut through the front part of the posterior tube cavities and through the tips of the anterior cavities.

FIG. 9. A still more anterior view, only the cross sections of the two deeper portions of the anterior cavities being in evidence.



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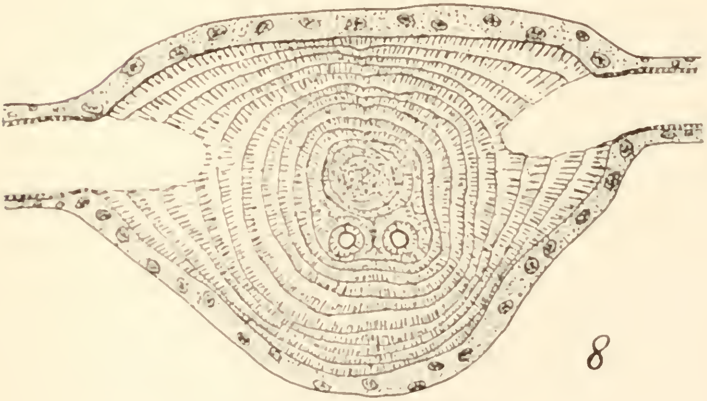

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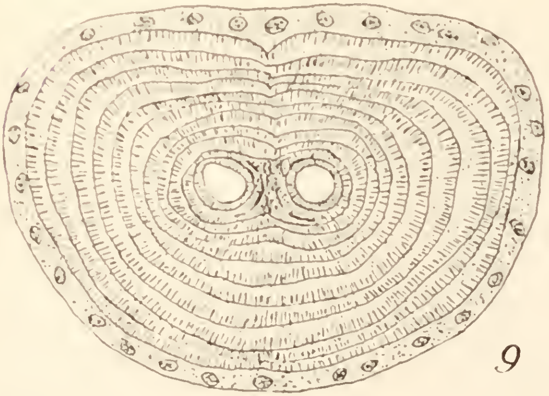

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THE EFFECTS OF SOME AMIDO-ACIDS ON THE DEVELOPMENT OF THE EGGS OF ARBACIA AND OF CHÆTOPTERUS.

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In 1909, Mathews published a short account of some experiments which he made to ascertain the effects of various amido-acids on the development of the eggs of *Arbacia*. The results of these experiments have considerable theoretical interest, since they seem to show that the course of embryonic development can be determined, to a greater or a less extent, by these products of protein digestion.

While I was working in the Marine Biological Laboratory at Woods Hole, Mass., in the summer of 1909, Dr. Mathews kindly furnished me with a number of amido-acids in order that I might repeat and extend his experiments and make a detailed study of the different types of larvæ that might be obtained. As it seemed worth while to determine whether amido-acids can alter the course of development in various kinds of eggs or whether they have a specific action on the eggs of *Arbacia*, the experiments were carried beyond the limits originally intended and were made with the eggs of an annelid, *Chætopterus pergamentaceus*, as well as with the eggs of the sea-urchin, *Arbacia punctulata*.

In addition to cystin, leucin and tyrosin, the three amido-acids which Mathews used in his experiments, both kinds of eggs were subjected to the action of glutamic acid, aspartic acid, asparagine, glycocoll and alanin. In each series of experiments eggs from two or more females were thoroughly mixed and then artificially fertilized in sea-water. As soon as the polar bodies had been extruded, approximately equal portions of the eggs were transferred into finger bowls which contained 100 c.c. of the solution to be tested. As a control by which to judge of the effects of the solutions, one portion of the eggs was allowed to develop in 100 c.c. of normal sea-water. The various experiments were made in a similar manner and the eggs were kept under like conditions of light and of temperature during their development in order that the results of the experiments might not be affected by environmental conditions other than those that were being studied.

A. EXPERIMENTS WITH THE EGGS OF *Arbacia punctulata*.

As the breeding season of *Arbacia* is near its close the latter part of July, only a small number of eggs suitable for experimental purposes could be obtained. All of the eggs used were presumably in a normal physiological condition, as at least 90 per cent. of those in the control cultures developed in a normal manner and became plutei.

In each series of experiments observations were made at frequent intervals on the living embryos. These observations were later supplemented by a microscopic study of various lots of material that had been fixed in corrosive sublimate and stained with Heidenhain's iron-hæmatoxylin or with Delafield's hæmatoxylin followed by eosin.

Cystin ($C_6H_{12}O_4N_2S_2$).—As this substance is very insoluble in cold sea-water, the solution used in the first experiment that was made was prepared in the following way: A quantity of the pure crystalline salt was placed in a flask of sea-water heated to 40° C. The mixture remained at this temperature for one half hour and was then sealed and set aside. After three days the solution was filtered, to remove the undissolved cystin, and used within a few hours.

A lot of *Arbacia* eggs was fertilized at 11.45 A.M. on the morning of July 14, 1909, and a portion of them was placed in the saturated solution of cystin at 12.15 P.M. These eggs were found to be segmenting in a normal manner when division of the eggs in the control culture took place at 12.50 P.M., and for some hours the eggs of both cultures seemed to be developing at about the same rate. If the cystin had any effect on the segmentation it was too slight to be detected either in the living eggs or in preserved material.

On the morning of July 15, both cultures contained many living embryos; those of the control were well-developed gastrulæ that were swimming at the surface of the water in a normal manner; those in the cystin solution were decidedly smaller than the control larvæ, and most of them were swimming at the bottom of the dish. Thirty hours after the experiment was started all of the larvæ in the cystin solution were dead, although the larvæ in the control culture were still in good condition. Preserved material showed that the development of the eggs that had been subjected to the action of the cystin solution took place in a perfectly normal manner, although it was somewhat slower than that of the eggs in the control lot.

Mathews found that cystin produced a decided acceleration in the development of the eggs of *Arbacia*, which was apparent from the fourth division on. The solution that he used was made as follows: "One hundred centimeters of sea-water were shaken for a moment with about a centigram of crystalline cystin and the mixture poured into a finger bowl with the undissolved cystin. The eggs, fertilized something less than an hour before, were then added and the eggs lay during development among the crystals of cystin at the bottom of the dish." As a solution made in this way is undoubtedly much weaker than that employed in my first experiment, it seemed probable that the opposing results obtained by Mathews and myself might be due to the difference in the strength of the solutions to which the eggs were subjected. The experiment was therefore repeated with a different lot of eggs, the solution of cystin that was used being prepared in the manner described by Mathews.

In this experiment, also, the development of the eggs appeared to progress at about the same rate in both the cystin culture and in the control. Some of the eggs in the cystin solution seemed to segment much more rapidly than others, and a very few of them developed at a faster rate than the major portion of the eggs in the control culture. A careful comparison between the two cultures, made at intervals of about one half hour during the entire day, failed to show any marked acceleration in the development of the great majority of the eggs in the cystin solution. Twenty hours after the experiment began swimming larvæ were found at the surface in both cultures, so in this instance the development of the blastulæ was not retarded by the cystin. The solution was ultimately harmful, however, as all of the larvæ in the cystin culture died within thirty-six hours, while those of the control developed into plutei that lived for several days. No unusual types of larvæ were noted among the living forms, and none were found in microscopic preparations of the older embryos.

The *Arbacia* eggs with which Mathews experimented were undoubtedly in a very different physiological condition from those that I used, for Mathews states that in the control lots for his experiments "hardly a pluteus was to be found and these few were generally abnormal." In both of my control cultures the great majority of the eggs formed normal plutei that lived for some days. With such a great difference in the lots of eggs experimented upon it is not surprising that the results do not agree, since the reaction of eggs to any external stimulus depends, to a considerable extent, upon the particular physiological conditions existing in the eggs at the time that the stimulus is applied.

Leucin ($C_6H_{13}NO_2$).—By the use of a weak solution of "impure leucin" Mathews changed the course of development of the eggs of *Arbacia* so that many of the embryos were totally unlike *Arbacia* larvæ. "In many, evagination of the entoderm instead of invagination, took place. A few developed a ciliated band in the shape of the star-fish bipinnaria. . . . Another form was perfectly spherical with a single ciliated band about the middle. It looked in its external form like a small trochophore." Unfortunately, it was not possible to obtain any of the impure leucin with which Mathews produced these remarkable forms of *Arbacia* larvæ, and the leucin with which I experimented was presumably pure.

Solutions of various strengths (2, 1, $\frac{1}{4}$, $\frac{1}{10}$ and $\frac{1}{30}$ per cent.) were used on batches of eggs that were fertilized at 11.30 A.M. on the morning of July 16, 1909. The eggs in all of the cultures began segmenting at the same time as those in the control lot, but the stronger solutions very soon proved toxic and greatly retarded development. None of the eggs in the 2 per cent. solution of leucin had developed beyond the 2-cell stage at the time that the great majority of the eggs in all of the other solutions, as well as in the control, were in the 8-cell stage. A solution of this strength, however, does not kill the eggs quickly, as twenty hours after the experiment began this culture contained a few ciliated larvæ that were much smaller, and less active, than those of the control lot. Within twenty-four hours all of the larvæ in the 2 per cent. solution of leucin were dead.

A microscopic examination was made of a large number of eggs taken from the 2 per cent. solution of leucin at different stages in their development. Many of the young eggs were abnormal in that there was an irregular distribution of the chromosomes to the poles of the segmentation-spindle or a very unequal division of the blastomeres. Such abnormal eggs evidently died before reaching the blastula stage, as nearly all of the older embryos that were examined were normal although somewhat smaller than those of the control culture. A few abnormal blastulæ were found among the older larvæ, but as these larvæ showed only such irregularities of form as may be found in individuals of almost every control culture of *Arbacia* larvæ developing in a small amount of sea-water under laboratory conditions, they could not be considered as due to the specific action of the leucin in changing the course of development.

The eggs in the 1 per cent. solution of leucin began to show the injurious effects of the solution after the first hour, and from this time on their development, although normal, lagged behind that of the control: the weaker solutions had apparently no effects on the early segmentation. The blastulæ in the control culture began moving about fifteen minutes sooner than the larvæ in the other cultures, so evidently all of the leucin solutions retarded development somewhat after the first two or three hours. Plutei that seemed perfectly normal, and that lived for several days, developed in all of the weaker solutions. An examination of a considerable number of these embryos, preserved at various stages in their development, failed to show any larvæ that were in any way comparable to the unusual types that Mathews obtained with impure leucin.

A second experiment was made with leucin on July 24, 1909. In this instance a solution of the strength of $\frac{1}{2}$ per cent. was employed, since stronger and weaker solutions do not alter the course of development. From the beginning of the experiment the segmentation of these eggs lagged behind that of the eggs in the control lot, and the retardation in development was fully as great as that produced by the 1 per cent. solution of leucin in the former series of experiments. Later the development of these eggs progressed at a more normal rate, and after seven hours the embryos appeared nearly as well developed, and fully as vigorous, as those in the control. The next morning larvæ were swimming at the surface in both cultures, but those in the leucin solution soon dropped to the bottom of the dish and began to disintegrate. Microscopic preparations showed that the very great majority of these larvæ were normal in every respect.

Mathews states that in the summer of 1908, when his experiments were made, the sea-urchin eggs showed in many instances the remarkable peculiarity, recorded by Mathews and Whitcher ('03), that "a large number of eggs while living for several days not forming plutei, or but a small per cent. of irregular plutei." The experiments which Mathews made to test the action of amido-acids on the development of the eggs of *Arbacia* were made therefore, wholly or in great part, on eggs that were in a peculiar physiological condition when experimented upon: whether they could be considered as normal is doubtful. The unusual types of larvæ that Mathews obtained by treating eggs with a weak solution of impure leucin were probably due to abnormal or unusual conditions existing in the eggs at the time of their fertilization, and not to the specific actions of leucin in changing the course of development. The effects of leucin on eggs of *Arbacia* that are in a normal physiological condition when fertilized depends chiefly upon the strength of the solution used: a strong solution retards development and causes the early death of the embryos: a weak solution permits of normal development at first and is toxic only after many hours.

Tyrosin ($C_9H_{11}NO_3$).—This substance is not very soluble in cold sea-water, and in order to obtain a solution of sufficient strength one gram of tyrosin crystals was put into 100 c.c. of sea-water and the mixture brought to the boiling point. The solution was then cooled to laboratory temperature, filtered, and used at once.

The early development of the eggs used in this experiment was normal, although slightly delayed. After twenty hours ciliated larvæ were present in great number in the solution, but they were moving feebly and beginning to show degenerative changes. Prepared material showed that tyrosin had retarded the development of the eggs but produced no abnormalities. These results agree with those obtained by Mathews in a similar experiment.

Glutamic Acid ($C_5H_9NO_4$).—Various solutions of this substance (1, $\frac{1}{2}$, $\frac{1}{10}$ and $\frac{1}{30}$ per cent.) were used on the eggs of *Arbacia*, and all of them proved to be injurious from the beginning of the experiment. The eggs placed in the stronger solutions (1 and $\frac{1}{2}$ per cent.) were killed at once. A few of the eggs subjected to the action of the $\frac{1}{10}$ per cent. solution began to segment in a normal manner, but none of them developed beyond the early stages of segmentation. The eggs in the $\frac{1}{30}$ per cent. solution continued to live for some time, but their development was very greatly retarded and stopped entirely when the gastrula stage was reached. Preparations of these eggs showed that the effects of the glutamic acid was to check development, not to produce unusual types of larvæ.

Aspartic Acid ($C_4H_7NO_4$).—This substance has a more deleterious action on the eggs of arbacia than has glutamic acid. All of the eggs placed in a 1 per cent. solution and in a $\frac{1}{2}$ per cent. solution were killed at once; those subjected to the action of a $\frac{1}{10}$ per cent. solution did not develop beyond the 2-cell stage. A solution of the strength of $\frac{1}{30}$ per cent. allowed a considerable number of the eggs to develop to the blastula stage, but segmentation was very irregular and much slower than that of the eggs in the control culture.

Preparations of various lots of eggs that had been treated with aspartic acid solutions showed abnormal conditions not found in any of the *Arbacia* eggs subjected to the action of other amido-acids. Most of the eggs that had been subjected to the action of a $\frac{1}{10}$ per cent. solution of aspartic acid for four hours before fixation were found to be still unsegmented, and many of them had been entered by several spermatozoa. Only one sperm-nucleus had fused with the egg-nucleus, however, and the segmentation-spindle that was formed usually appeared normal, although in many cases it occupied a very eccentric position close to the periphery of the egg. All of the accessory spermatozoa at this time were in the form of a small, rounded nuclei that were scattered throughout the cytoplasm.

The $\frac{1}{30}$ per cent. solution of aspartic acid had a different action on different eggs, depending, doubtless, upon the condition of the eggs when they were placed in the solution. Five hours after the experiment was begun about one fourth of the eggs were still unsegmented; some of the eggs were just beginning to segment; while others were in later stages of segmentation, and the cleavage planes were coming in very irregularly in many cases. A very few eggs had reached the blastula stage at this time, but they were not as well developed as the eggs in the control lot. After twenty-two hours the number of eggs that had reached the blastula stage was found to be consideraly increased. Development had been checked by this time, however, and the greater number of larvæ appeared as more or less irregular masses of cells that were beginning to disintegrate.

Preparations of this material showed many cases of polyspermy. Some of the unsegmented eggs contained a large multipolar segmentation-spindle formed, evidently, by the fusion of several sperm-nuclei with the egg-nucleus: other eggs contained a segmentation-spindle of the normal size with the chromosomes very unequally distributed to the spindle poles. The condition of these eggs greatly resembled that which O. and R. Hertwig ('87) found could be induced in fertilized echinoderm eggs by subjecting them to the action of various chemical substances which prevented their normal development.

Asparagine ($C_4H_{10}N_2O_4$).—This amide of aspartic acid proved to be far less injurious to the eggs of *Arbacia* than did the latter substance, when used in solutions of the same strength (1, $\frac{1}{2}$, $\frac{1}{10}$ and $\frac{1}{30}$ per cent.). The great majority of the eggs in all of the cultures began to segment at the normal time and in a normal manner. After two hours the eggs in the 1 per cent. solution showed evidence of retarded development, but the eggs in all of the other solutions developed at a normal rate for some hours.

Twenty-four hours after the experiment began, ciliated larvæ were present in great numbers in all of the solutions, but they all died many hours before the death of the larvæ in the control culture.

Glycocoll ($C_2H_5NO_2$).—This substance, which is the simplest of the amido-acids, was much less harmful to the eggs of *Arbacia* than were any of the other amido-acids used in these experiments. During the first twenty-four hours the development of the eggs did not appear to be affected in any way by the solutions used (1, $\frac{1}{2}$, $\frac{1}{10}$ and $\frac{1}{30}$ per cent.), but during the second day the embryos began to show degenerative changes, and all of them died about fifty hours after the experiment began. Sections of these eggs fixed at various stages of development merely confirmed the observations on the living forms, as no unusual types of larvæ were found.

Alanin ($C_3H_7NO_4$).—This amido-acid dissolves readily in cold sea-water, and it was used in solutions of the following strengths: 2, 1, $\frac{1}{2}$, $\frac{1}{10}$ and $\frac{1}{30}$ per cent. The stronger solutions (2, 1 and $\frac{1}{2}$ per cent.) retarded development from the beginning: the weaker solutions had no apparent effects on the segmentation of the eggs. After twenty-four hours each of the solutions contained a large number of swimming larvæ, and only those in the 2 per cent. solution showed any evidence of retarded development. The embryos in all of the cultures died some hours before the death of the control larvæ, so weak solutions of alanin cannot be considered as favorable media in which to rear the eggs of *Arbacia*. Preserved material showed no abnormalities worthy of note at any stage of development.

All of the amido-acids used in this series of experiments with the eggs of *Arbacia* proved to be toxic, the injurious effects of any substance depending very largely upon the strength of the solution used. In no case was the course of development altered in a definite direction, except in the very young eggs and in these the abnormalities produced were of the types commonly found when fertilized eggs of the sea-urchin are treated with various chemical solutions.

B. Experiments with the Eggs of *Chætopterus pergamentaceus.*

As the eggs of *Chætopterus* could be obtained in considerable numbers at Woods Hole in the summer of 1909, experiments were made to study the influence of amido-acids on the early development of this annelid, in the hope that some definite alterations in development might be produced comparable to those obtained by Loeb ('01) and by Lillie ('02) when eggs of *Chætopterus* were treated with potassium salts. Material intended for microscopic study was preserved in Boveri's picric-acetic solution and stained with hæmatoxylin.

Cystin.—On the morning of August 6, 1909, a lot of *Chætopterus* eggs was placed in 100 c.c. of a saturated solution of cystin as soon as the polar bodies had been extruded. The early development of these eggs was slightly accelerated, and swimming larvæ were found in this culture nearly one half hour before any movement could be detected in the control larvæ. The next day the cystin solution was swarming with well-developed trochophores, but they all died about fifty hours after the experiment began. No abnormal embryos were noted at any stages of development and none were found in preserved material.

The experiment was repeated several days later with eggs from another female. The results obtained were practically the same as in the first experiment, since there was more rapid development during the segmentation period. The solution proved to be toxic after thirty hours, however, killing the embryos without producing any alterations in structure.

Leucin.—In one series of experiments this substance was used on the eggs of *Chætopterus* in solutions of the following strengths: $\frac{1}{2}$, $\frac{1}{10}$ and $\frac{1}{30}$ per cent. None of these solutions had any marked effects on the early segmentation of the eggs, but they evidently caused a slight acceleration in development during a later period as the larvæ in all of the solutions began moving some thirty minutes before there was any movement of the control larvæ. Twenty hours after the experiments were started all of the cultures were carefully examined. The majority of the eggs that had been treated with the $\frac{1}{2}$ per cent. solution had stopped their development in the blastula stage, and were lying at the bottom of the dish apparently dead; a very few larvæ were swimming at the surface of the solution, but they had evidently reached their maximum development and would soon die. The $\frac{1}{10}$ per cent. solution contained a considerable number of swimming larvæ, but these larvæ were not in good condition and plainly showed the injurious effects of the leucin. A large number of ciliated embryos were found in the $\frac{1}{30}$ per cent. solution, and they appeared somewhat further advanced in development than those in the control culture. Degenerative changes appeared in these larvæ in about twenty-four hours, however, and all of them were dead within thirty hours. No unusual types of larvæ were found in preparations of these eggs fixed at various stages in their development.

As it seemed possible that the solutions of leucin employed in the experiments described above might have been too weak to produce any alteration in the development of the eggs, a second experiment was made in which a batch of eggs was subjected to the action of a 1 per cent. solution of leucin. These eggs segmented at the normal time, but two hours later their development was found to be lagging behind that of the eggs in the control culture. After four hours the retardation in development was very marked, and in some instances two or more eggs had fused together. Loeb and Lillie have noted that the fusion of several embryos into giant forms is a phenomenon of frequent occurrence when eggs of *Chætopterus* are treated with potassium salts. In twenty hours all of the larvæ were dead, and so disintegrated that it was impossible to preserve any material fit for study. Sections of eggs fixed in earlier stages of development failed to show any abnormalities except the occasional fusion of two or more embryos.

Tyrosin.—This substance was used on the eggs of *Chætopterus* in a saturated solution which is less than $\frac{1}{10}$ per cent. Only a very few of the eggs had segmented when the first division occurred in the control eggs. After four hours the tyrosin culture showed all stages in development from the unsegmented egg through to late segmentation, the most advanced eggs being apparently at the same stage of development as the eggs of the control. All of the embryos in the tyrosin solution died within twenty-four hours after the experiment was started. Preserved material showed that tyrosin acts on the eggs of *Chætopterus* as it does on the eggs of *Arbacia*, causing a marked retardation in development but producing no specific abnormalities.

Glutamic Acid.—Solutions of various strengths (1, $\frac{1}{2}$ and $\frac{1}{10}$ per cent.) were used, the eggs being placed in the solutions about three quarters of an hour after their fertilization. All of the eggs in the two stronger solutions were evidently killed at once as none of them made any attempts to divide. Some of the eggs in the $\frac{1}{10}$ per cent. solution began to elongate after the solution had acted upon them for one hour, and later many of these eggs took on an irregular shape as if attempting to divide into several cells at the same time. None of these eggs had segmented after five hours, however, so they were all returned to normal sea-water in the hope that they would then be able to continue their development. There was no segmentation of any of the eggs, although they appeared to live for some hours.

Sections of preserved material showed that the segmentation-spindle had formed in many eggs in an apparently normal manner, but that development had been stopped at this point.

Aspartic Acid.—Eggs of *Chætopterus* fertilized at 10.55 A.M. on August 8, 1909, were placed in solutions of aspartic acid (1, $\frac{1}{2}$, $\frac{1}{10}$ and $\frac{1}{30}$ per cent.) at 11.25 A.M. The eggs in the control culture were segmenting at 11.55 A.M., but no evidence of cleavage could be detected in any of the eggs in the aspartic acid solutions until 1.30 P.M., when a few of the eggs in the $\frac{1}{30}$ per cent. solution began to elongate as if about to divide. A number of these elongated eggs were isolated and carefully watched for some time, but in no case did any division occur. Sections of preserved material showed that some eggs contained a normal segmentation-spindle, while others had a multipolar spindle that occupied an eccentric position close to the periphery. The stronger solutions of aspartic acid killed the eggs before the formation of the segmentation-spindle.

Asparagine.—Solutions of this substance of the same strengths as those used in the experiments with aspartic acid were tested. Normal cleavage began in the eggs of all of the cultures at the same time as in those of the control lot. Observations made at frequent intervals during the next four hours showed that segmentation was progressing in a normal manner and at about the same rate in all of the solutions.

Five hours after the eggs had been fertilized a few larvæ in the $\frac{1}{10}$ per cent. solution were moving slowly: at this time there was no movement of any of the embryos in the other cultures or in the control lot. A weak solution of asparagine, therefore, slightly accelerates the development of the eggs of *Chætopterus*, if it be that an earlier movement of the embryos is indicative of a more advanced stage of development. At the end of the sixth hour the effects of the various solutions were very marked: the embryos in the $\frac{1}{10}$ per cent. solution were moving more actively than those in the control, and they seemed slightly better developed; the larvæ in the other solutions were moving slowly and their development lagged considerably behind that of the control larvæ. After eight hours the larvæ in the 1 per cent. solution were all at the bottom of the dish and evidently dying; no abnormal types of larvæ could be detected among the living forms, and none were found in preserved material that was examined later. The embryos in the other solutions were swimming at the surface after ten hours, but none of them lived more than twenty-four hours.

Glycocoll.—In the strengths of solutions used (1, $\frac{1}{2}$ and $\frac{1}{10}$ per cent.), this substance did not appear to have any effects whatever on the eggs during the first twelve hours. On the second day the larvæ began dying, and all of them had been killed by the end of the third day.

Alanin.—Batches of *Chætopterus* eggs that had been artificially fertilized at 10.30 A M. on the morning of August 8, 1909, were put into various solutions of alanin (1, $\frac{1}{2}$, $\frac{1}{10}$ and $\frac{1}{30}$ per cent.) at 11 o'clock. The eggs in all of the cultures, including the control, began segmenting at the same time, and all of them developed at about the same rate during the next two hours. At 3.30 P.M. a number of swimming larvæ were found in the $\frac{1}{10}$ and in the $\frac{1}{30}$ per cent. solutions, but at this time there was no movement of the larvæ in any of the other cultures. At 4.30 P.M. ciliated larvæ were present in great numbers in all of the solutions; but the larvæ in the 1 per cent. solution could move but slowly, and soon all of them sank to the bottom of the dish and disintegrated.

At 9 A.M. on the morning of August 9, the larvæ in the $\frac{1}{2}$ per cent. solution were dying, and a number of giant embryos had been formed by the fusion of two or more of the larvæ: the embryos in the $\frac{1}{10}$ per cent. and in the $\frac{1}{30}$ per cent. solutions were apparently normal and were moving vigorously. All of the larvæ were dead on the morning of August 10, although the trochophores in the control culture were still very active at this time. Preserved material showed no abnormalities worthy of note.

As weak solutions of alanin did not seem to affect the early development of the eggs adversely a second series of experiments was made in which batches of *Chætopterus* eggs were treated with 4 per cent. and with 2 per cent. solutions of alanin as soon a they had extruded their polar bodies.

None of the eggs in the 4 per cent. solution segmented, and sections of preserved material showed that the eggs had been killed before the formation of the segmentation-spindle. When cleavage began in the eggs of the control lot at 11 A.M. a very few of the eggs in the 2 per cent. solution were dividing in an apparently normal manner; in the great majority of the eggs segmentation was very greatly delayed. After four hours only a few eggs had reached the 4-cell stage, and in these eggs the cleavage planes had come in very irregularly. An hour later development had stopped entirely and the eggs were fusing into large, irregularly shaped masses. At this time the eggs were transferred into normal sea-water in the hope that segmentation might be resumed, but although the eggs seemed to live for some hours, none of them developed beyond the 4-cell stage.

In microscopic preparations of eggs that had been in the 2 per cent. solution of alanin for two hours before fixation only a very few normal 2-cell stages were found, and the great majority of the eggs contained a multipolar spindle with the chromosomes very irregularly distributed along the spindle fibres. Material fixed after the solution had acted for five hours showed that only the first cleavage in any of the eggs was normal and that in most eggs development had stopped at this point. Where further division had occurred the blastomeres were very irregular in size and shape, and although hundreds of eggs were examined no stage later than an 8-cell stage could be found.

When multipolar spindles formed in the eggs as a result of their treatment with a 2 per cent. solution of alanin the eggs, apparently, were never able to divide, although there seemed to be a long period during which active and resting stages alternated with each other. In the resting stages the eggs contained either one large, oblong nucleus, or several smaller ones that were more or less irregular in outline. In the active periods one large, multipolar spindle with hundreds of chromosomes scattered about it would be formed, or several small spindles, all more or less irregular, would be scattered throughout the cell. In some of these eggs a number of accessory asters were formed, similar to those that Morgan ('96, '99) found could be produced in the eggs of *Arbacia* and of various other forms by means of salt solutions.

A 2 per cent. solution of alanin produced greater abnormalities in the eggs of *Chætopterus* than did any of the other solutions of amido-acids that were used, but as these abnormalities were of the types that can be produced in different kinds of eggs by treatment with various salts they cannot be considered as the result of any specific action on the part of the alanin.

SUMMARY AND CONCLUSIONS.

With the exception of cystin, which is a sulphur-containing compound, all of the amido-acids used in these experiments are composed of the same chemical elements, yet they differ to a marked extent in their toxic action on developing eggs. Glutamic acid and aspartic acid are by far the most injurious, even a $\frac{1}{30}$ per cent. solution of these substances killing the eggs of both *Arbacia* and of *Chætopterus* at a very early period. Glycocoll, on the other hand, permits of the development of normal plutei and trochophores, and only injures the embryos after twenty-four hours. The other amido-acids used retard development, to a greater or less extent, depending chiefly upon the strength of the solution employed.

A brief summary of the effects of the various solutions of amido-acids on the development of the eggs of *Arbacia* and of *Chætopterus* during the first twelve hours is given in the following table. Ultimately all of the solutions are toxic, even though they appear to favor development during an early period.

Table I.

Amido-acid.	Solution Used.	Effects on *Arbacia* Eggs.	Effects on *Chætopterus* Eggs
Cystin	Saturated	No effects on segmentation; later development retarded.	Development accelerated.
Leucin.	$\frac{1}{30}$ per cent.	Development very slightly retarded.	Development slightly accelerated.
	$\frac{1}{10}$ per cent.	Development very slightly retarded.	Development slightly accelerated.
	$\frac{1}{4}$ per cent.	Development very slightly retarded.	....
	$\frac{1}{2}$ per cent.	Development slightly retarded.	Development accelerated at first, but stopped in blastula stage.
	1 per cent.	Development retarded after 1 hour.	Development retarded after 2 hours; embryos fused.
	2 per cent.	Development greatly retarded; a few eggs abnormal.	....
Tyrosin.	Saturated.	Development retarded.	Development retarded.
Glutamic acid.	$\frac{1}{30}$ per cent.	Development stopped in the gastrula stage.	....
	$\frac{1}{10}$ per cent.	Eggs killed in early segmentation.	Eggs lived for some time, but no segmentation.
	$\frac{1}{2}$ per cent.	Eggs killed at once.	Eggs killed at once.
	1 per cent.	Eggs killed at once.	Eggs killed at once.
Aspartic acid.	$\frac{1}{30}$ per cent.	Development stopped in blastula stage; many eggs abnormal.	Eggs lived for some time, but no segmentation.
	$\frac{1}{40}$ per cent.	Development stopped at 2-cell stage; many eggs abnormal.	Eggs killed at once.
	$\frac{1}{2}$ per cent.	Eggs killed at once.	Eggs killed at once.
	1 per cent.	Eggs killed at once.	Eggs killed at once.
Asparagine.	$\frac{1}{30}$ per cent.	No effects noted.	....
	$\frac{1}{10}$ per cent.	No effects notes.	Development slightly accelerated.
	$\frac{1}{2}$ per cent.	No effects noted.	Segmentation not affected; later development retarded.
	1 per cent.	Development retarded after 2 hours.	Segmentation not affected; later development retarded.
Glycocoll.	$\frac{1}{30}$ per cent.	No effects noted.	No effects noted.
	$\frac{1}{10}$ per cent.	No effects noted.	No effects noted.
	$\frac{1}{2}$ per cent.	No effects noted.	No effects noted.
	1 per cent.	No effects noted.	No effects noted.
Alanin.	$\frac{1}{30}$ per cent.	No effects noted.	Development slightly accelerated.
	$\frac{1}{10}$ per cent.	No effects noted.	Development slightly accelerated.
	$\frac{1}{2}$ per cent.	Development somewhat retarded.	Segmentation not affected; older embryos fused.

Amido-acid.	Solution Used.	Effects on *Arbacia* Eggs.	Effects on *Chætopterus* Eggs.
Alanin.	1 per cent.	Development greatly retarded.	Development retarded after 2 hours.
	2 per cent.	Development greatly retarded.	Development retarded; many eggs abnormal.
	4 per cent.	....	Eggs killed at once.

As shown in the above table, all of the stronger solutions of amido-acids that were used had much the same effect on both kinds of eggs experimented upon, but several of the weaker solutions had a much more pronounced action on the eggs of *Chætopterus* than on those of *Arbacia*. Weak solutions of cystin, of leucin, of asparagine and of alanin accelerate the development of the eggs of *Chætopterus* to a noticeable extent, yet none of these solutions have apparently any effect on the early development of the eggs of *Arbacia*. The eggs of *Chætopterus* cannot segment at all when placed in a $\frac{1}{30}$ per cent. solution of aspartic acid, although this solution permits the eggs of *Arbacia* to develop to the blastula stage.

The abnormalities produced in the eggs of *Arbacia* and of *Chætopterus* by various solutions of amido-acids consist chiefly of polyspermy, irregularities in the mitotic figures, variable cleavage, and a fusion of several embryos into giant forms. No embryos were found that showed either the larval characteristics of other forms or marked peculiarities of structure that might be attributed to the specific action of the solution in which they were reared.

The results obtained in these experiments indicate that solutions of amido-acids can alter the rate at which the eggs of *Arbacia* and of *Chætopterus* develop, but that they have no influence whatever in determining the character of the development, when the eggs experimented upon are in a normal physiological condition.

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A DISCUSSION OF CYCLOPS VIRIDIS JURINE.

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Cyclops viridis Jurine, or, as it was formerly called, *Cyclops brevicornis* Claus, the object of the classic researches of Haecker, is described as being represented in North America in the form of several varieties.

C. Dwight Marsh ('10), in his revision of the North American species of *Cyclops*, divides the species *viridis* into four varieties: var. *ingens* Herrick, var. *brevispinosus* Herrick, var. *parcus* Herrick, and var. *Americanus* Marsh.

Ingens includes the largest forms of the species and possibly corresponds to the European var. *gigas* Claus.

Americanus is the most abundant variety of *viridis* in American waters. It is to be met with in almost any ditch or small pond. *Parcus* is much more local in its haunts but in the localities where it is to be found it may be abundantly represented. I have never found the two varieties together. *Parcus* is, on the average, smaller than *Americanus*.

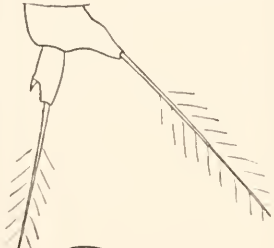
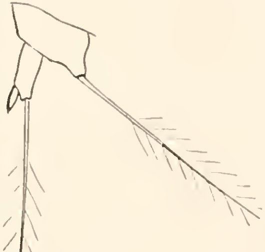
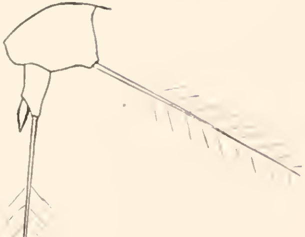
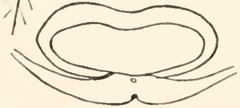
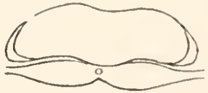
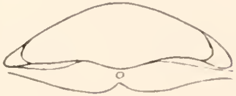
In the accompanying table I have indicated the main features distinguishing the European and the two last mentioned American varieties.

The inner margin of the furcal rami of the tail is never ciliated in the two American varieties as it is in the European form.

The rudimentary fifth thoracic appendage of the European *viridis* (see table) is described by Schmeil ('92) as having the small spine on the inner margin of the second segment either a mere cuticular protuberance or connected with that segment by a distinct joint. The latter feature is characteristic of the two American varieties (see table).

The number of spines on the terminal segments of the outer rami of the four swimming appendages in the European *viridis* and in *parcus* are two for the first pair of appendages and three

Cyclops viridis Jurine (excl. var. *gigas*, *ingens*, and *brevispinosus*).

	European Variety.	Var. *parcus* Herrick.	Var. *americanus* Marsh.
1. Fifth foot (that of the Eur. var. is drawn on a scale half of that of the other two).			
2. Receptaculum seminis (that of Eur. var. from Claus).			
3. Inner margin of furcal rami.	Ciliated.	Devoid of cilia.	Devoid of cilia.
4. Spine formula.	2, 3, 3, 3.	2, 3, 3, 3.	3, 4, 4, 4.
5. Average body-length.	2.2 mm. (Wolf)	1.4 mm.	1.5 mm.
6. Chromosome number.	12	6	10

for the next three pairs. *Americanus* has three on the first and four on the other three pairs.

E. F. Byrnes ('09) considers *parcus* and *Americanus* to be heterogenous[1] forms of the same species. This she assumes from the fact that the only fundamental difference noted between them is the armature of the swimming appendages and this appears to be variable, for occasionally one may find an adult *Cyclops* unmistakably *C. Americanus*, in which most of the swimming feet agree with *C. Americanus* in having four spines on the terminal segments of the outer rami, while others are in the condition characteristic of *C. parcus*, having but three spines on the terminal segments of the rami.

Neither Byrnes nor Marsh ('10) have remarked upon the dissimilarity in the shape of the receptaculum seminis of the two forms. Systematists working on Cyclopidæ admit that the most constant and characteristic feature for a given species is the shape of that organ. It is remarkable, therefore, that more care is not taken in figuring the seminal receptacle of described forms.

In all three varieties the receptaculum consists of a larger antero-median portion and two narrower postero-ventral portions which are carried out laterally as the sperm ducts.

Parcus possesses a receptaculum (see table) which resembles that of the European *viridis* in that the upper portion is concave anteriorly. That of *Americanus* (see table) is convex.

Another point which seems to leave no room for doubt as to the distinctness of the two varieties is the constant difference in their chromosome number. I have found that the somatic chromosome number in *C. Americanus* is 10 whereas in *C. parcus* it is 6.

Specimens collected in widely separated localities, as Toronto, Woods Hole, and New York, have thus far shown this difference in chromosome number to be constant for the two varieties.

A specimen taken from a pure culture of *C. parcus* showed a variation in the spines of its thoracic limbs, the spine formula being 3, 4, 4, 3 or 4 (?). A systematist would probably consider this a case of interrelationship between *C. parcus* and *C. Americanus*. That this cannot be so is to be seen from the following: I was fortunate enough to section the specimen when the chromosomes of its oviduct eggs were in the so-called "biserial arrangement" and where the count is particularly easy. The presence of *three pairs* of chromosomes (see text figure) leaves no doubt as to the identity of the specimen.

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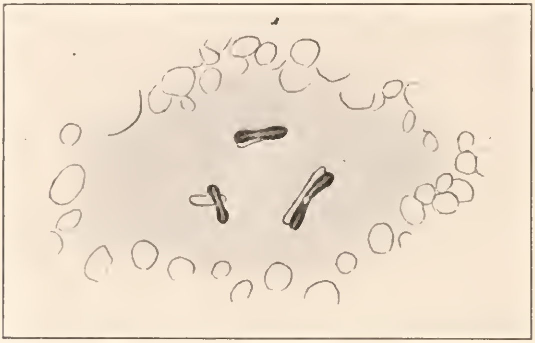
[1] The term *heterogeny* is used here to denote the existence of two adult forms which represent successive generations, both of which are sexually mature, but morphologically unlike.



Nucleus of oviduct egg of *Cyclops parcus*, showing the three pairs of chromosomes in "biserial arrangement."

The European *Cycl. viridis* has 12 chromosomes (Haecker, Braun).

Haecker ('97) described *Cyclops brevicornis* Claus (*viridis* Jurine) which he studied as being anywhere from 3.5–5 mm. in length. The size mentioned indicates that he was probably working with var. *gigas* Claus. It is remarkable that he gives the somatic chromosome number to be 24, although in the ovary he describes the chromosomes as bivalent, being 12 in number.

Braun ('09) also studied *Cyclops viridis* Jurine but not the variety *gigas*. He gives the somatic number of chromosomes for the typical species as 12. Unfortunately he does not mention sizes except in stating that the species varies between 1.5–5.1 mm. in length.

Schmeil ('92) gives the body length of the typical European *viridis* to be anywhere from 1.5–3.5 mm.

Wolf ('05) places the average length at 2.2 mm. Our American *parcus* on the other hand is not more than half the average size of the European form. The specimens I have met with have never been over 1.5 mm. in length and are much more frequently between 1.2 mm. and 1.4 mm. long.

The drawings in the accompanying plate also show the disparity in size between the American and the European forms, that of the fifth foot of the European variety, from a specimen in my possession, being drawn to a scale half of that of the other drawings.

Is it not possible that we have here a case similar to that which R. R. Gates ('09) discovered in *Œnothera? Œnothera gigas*, a giant mutant of *O. Lamarckiana*, was found to possess 28 chromosomes or double the number of the parent form (14). Its cells were found to be correspondingly larger. Gates suggested that in an egg of *O. Lamarckiana* a double number of chromosomes arose from a division of the chromosomes unaccompanied by nuclear and cell division soon after fertilization and that this egg developed into the *O. gigas* form.

Either *C. parcus* or the European *C. viridis* may conceivably be a mutation one of the other if, in the one case, all of the chromosomes split into halves without subsequent nuclear division, or, in the other, go into mitosis without splitting so as to produce the number 12 for the European *viridis* or 6 for the American *parcus*. The cells of the European *viridis* containing twelve chromosomes would then be twice the size of those of *C. parcus* which has only six. The actual discrepancy in size between the two forms could thus be explained.

Note.—Since the above was sent to the printers I have secured specimens of a *Cyclops viridis* from several pools near Edgewater, N. J. They are mostly from 2–3 mm. in length although several mature individuals measure only 1.4 mm.

Not only do they resemble the typical European *viridis* Jurine in size; in the ciliated inner margin of the furcal rami; in the fifth foot with the very small barely jointed spine on the second segment; but also in the spine formula for the four swimming feet which is 2, 3, 3, 3; and, most significant of all, in the shape of the seminal receptacle, the figure shown in the table for the European *viridis* being an exact picture of the same organ in the form under discussion.

And, lastly, the somatic chromosome number I have found to be 12, the same as that of the European *viridis*.

I see no reason why this form should not be entitled to the exclusive rights of the name *Cyclops viridis* Jurine; and *C. Americanus*, *C. parcus*, and *C. brevispinosus*, each with its distinctive chromosome number, spinal armature for the swimming feet, and seminal receptacle, be raised to the rank of separate species.

That the individuals I have just secured are not to be classed with *C. ingens* Herrick I conclude from Herrick's statement ('95) that the latter is merely an exaggerated form of *C. Americanus*. Neither are they to be compared with the forms which Miss Byrnes describes as *C. ingens* (?) for this latter species Miss Byrnes distinctly states as possessing the *brevispinosus* spinal armature of the swimming feet. My individuals, on the other hand, possess the *parcus* spinal armature for both the outer and inner rami of the swimming feet, and this armature is identical with that of the European *viridis*.

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NOTES ON THE HISTORY OF BARRED BREEDS OF POULTRY.[1]

RAYMOND PEARL.

At the present time modern breeds of poultry are being much used as material for the study of various problems in genetics. On this account matters connected with the early history of these breeds take on a general biological interest which they would not otherwise possess. It is the purpose of these notes to call attention to certain historical matters which bear directly upon some problems now occupying the writer's attention.

I. The Production of the Barred Color Pattern from the Mating of Solid Black with Solid White Birds.

In an earlier paper[2] from this laboratory the following statement was made respecting the origin of the barred color pattern, seen now in Barred Plymouth Rock and several other kinds of poultry.

"In regard to the origin of this barred color pattern in poultry very little can be learned. It is known that the Barred Plymouth Rocks owe their barring to the Dominique, which was used in the crossing which led to the production of this breed. But as to the source of the barring in the Dominiques we have found no evidence. Wright (1) says (p. 301) that: 'The colour (barring) itself is not a primary one, but the produce of white with either black or a very dark color. Such colours, mated together, produce as the result, on a wide average of cases, more or less of blacks, whites, mottles or splashes with the plumage of Houdans and Anconas, blues or blue duns like that of blue Langshans and Andalusians, and that bluish barred plumage known as Dominique in America and Cuckoo in England. When once produced, this last colour has however a strong tendency to permanence; and in the common native Dominique fowl of the

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[1] Papers from the Biological Laboratory of the Maine Experiment Station, No. 36.

[2] Pearl, R., and Surface, F. M. "On the Inheritance of the Barred Color Pattern in Poultry." *Arch. für Entwicklungsmech.*, Bd. XXX. (Teil I.), pp. 45–61, 1910.

West Indies and United States it had been preserved and bred so long as to be of a very fixed type indeed, though even in these fowls there was a constant tendency for white or black feathers of the original components to appear, as well as the straw or red which always troubles breeders of white or black fowls.'

"This is the statement of a fancier, made without special study of the inheritance of barring. It is certainly correct in the statement that the barring has become firmly fixed in the Plymouth Rock at least. Such a thing as a completely non-barred bird appearing in any 'pure bred' strain of Barred Plymouth Rocks no longer occurs and has not for a number of years. The statement that the barred pattern originated from a cross between black and white birds, as a sort of intermediate condition, is, we feel certain, a mistake. The barring is a perfectly definite pattern, not simply a mixture of black and white, or a 'splashed' coloration such as is seen in Houdans. The inheritance of barring is of such character as to indicate most strongly that we have to deal here with a unit character, viz., a particular definite and characteristic pattern. Further, so far as we are aware, none of the experiments regarding the inheritance of color in poultry carried out by Bateson, Punnett, Hurst, Davenport[1] or the present writers give the slightest evidence that breeding black and white birds together will produce barred offspring. Finally, in the case of the Plymouth Rocks, where this pattern reaches its most perfect expression, the known history of the breed makes it certain that the barring was not created *de novo*, but was taken from the Dominique."

Since this was written I have found in the literature an interesting piece of definite circumstantial evidence regarding the appearance of the barred pattern in the offspring of a solid black and solid white bird mated together. This would seem at first glance to be clear proof for the *de novo* origin of the pattern. As such it is worth discussing. The case in point concerns the origin of race of bantam fowls known among English fanciers as Cuckoo Pekins. The Cuckoo Pekins were originated about 25 years ago by the well-known English fancier and authority on the bantam breeds, Mr. William Flamank Entwisle. In his book[1] on bantams, which is the standard work on the subject in English, he gives the following statement regarding the appearance of the Cuckoo variety (*loc. cit.*, p. 40).

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[1] It should have been stated that Davenport (Carnegie Institution, Publication 52, p. 40) has reported barred offspring from crossing a White Leghorn bantam ♂ with a Black Cochin bantam ♀. His results from pure matings, however, show plainly, as he himself states (*loc. cit.*, p. 40 and p. 75), that the White Leghorn stock used carried the barred pattern factor. This case then evidently has no critical bearing on the point under discussion here.

"We now pass on to the Cuckoo Pekins. These are a very recent introduction; in fact, the first time that a pair of this variety was exhibited was at the Bawtry Show in September, 1888, when Master Frank E. Entwisle exhibited three pairs of them, which produced quite a sensation, one pair winning the silver cup. In colour they are quite as perfect as any shown since, though they are now much improved in shape, cushion, softness of tail, and abundance of foot and shank feather. We first produced Cuckoos in this way: While crossing Black Pekins and White Booted with the double intention of strengthening the Blacks, and producing White Pekins, we reared, amongst others, one a rather dirty looking white, so very excellent in shape, etc., that we thought it good enough to show as White Pekin at the Dairy Show; so we had it caught and washed, but to our surprise it would not come a better white than when first put into the soapsuds; we tried a thorough good soaking, washing, and rinsing, and then had her carefully dried; and on the following morning we had a careful look at her, when we discovered faint but regular bars of stone colour, on a milk white ground. We at once saw that in this pullet we had a more valuable prize than a pure white would have been, and we mated her with her sire, a Black Pekin cock, for the next season. From this mating we had distinct cuckoo markings, and these pullets we mated with a Cuckoo cockerel, which Mr. Leno kindly sent us, and which he bred from his imported Chinese Cuckoo cock, we believe the only one ever sent from China. Then we bred in-and-in, and back to the pure Black Pekins, until they have proved themselves capable of, now and then, beating all other colours of Pekins."

In considering this case the first point to be noted is that there is every reason to suppose that entire reliance may be placed on the statements made, so far as they go. In other words, Mr. Entwisle may be regarded a reliable witness as to the facts. He held a distinguished place among British fanciers, and his book furnishes much evidence that he was a keen and careful observer. Of course, as is usual in such fanciers' reports, critical evidence is lacking at important points in the case here under discussion.

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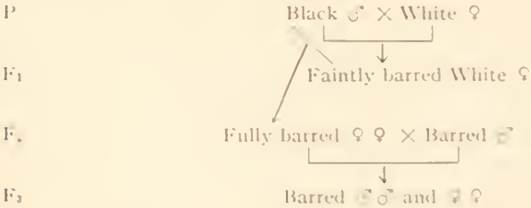
[1] Entwisle, W. F., "Bantams." Wakefield, 1894 (?), pp. 1–116.

One thing, however, seems clear, namely that while in the particular instance cited, the barred pattern appeared in the F_1 generation from a mating of black by white, it is by no means certain that one or the other of the parents did not carry barring latent (*i. e.*, as a cryptomere). The difficulty in taking this case as proof of the *de novo* origin of the barring lies in the fact that a "Mr. Leno" had at that time a cuckoo cock "imported from China," which evidently carried the barred pattern in hereditary form. If this were the case it is obvious that other color varieties of Asiatic bantams might carry the barred pattern determiner or factor in their gametes, without its being somatically visible. That this is the true explanation of the case is indicated by the fact that in this pullet the *pattern* appeared at once in apparently almost perfect condition ("faint but *regular* bars"). This would scarcely be expected if what is occurring here is the beginning of the synthesis of a barred pattern from pure black and white. Rather one would suppose that at the outstart the barring would be irregular and indefinite in character.

This case described by Entwisle must, then, be regarded as failing to furnish *critical* evidence of the *de novo* origin of the barred pattern in fowls from crossing solid black and solid white.

Incomplete as are the data, however, the case is of interest in another direction. If it be assumed, for the reasons set forth above, that one of the parents of this faintly barred pullet carried the gametic determiner for barring, then one must conclude that it was the White Booted parent. The reasons are, (*a*) that in all cases now known at least (and they cover in published and unpublished work a fair number of different breeds of poultry), if the factor[1] for black pigment and the factor for the barred pattern are present together the zygote will show the barred character. In other words there is no evidence that a black bird can carry barring latent. If such a bird has the barring factor at all it will be visible somatically, so far as present experience goes. (*b*) The white of the White Booted ♀ parent must have been "dominant white," from the account given. This is indicated by the fact that the prevailing color of the F_1 bird from the cross of this white ♀ with a black ♂ was white. A "dominant white" bird could carry barring gametically for an indefinite period without its showing somatically.

From the data given it is possible to construct the following pedigree.



Now in view of the fact that the barred pattern in all cases so far studied behaves as a sex-correlated character for which the female is heterozygous, this pedigree gives a remarkable result. If the barring was latent in the original White Booted ♀, which seems probable for the reasons set forth above, the observed results *can only be explained on the assumption that the factor for barring and the female sex determiner were carried in the same gamete*. Thus if *B* denotes presence of factor for barred pattern; *W* presence of factor for "dominant white"; *N* presence of factor for black; *F* presence of factor for ♀ sex, and the corresponding small letters the absence of these factors, the pedigree for this black × white cross would stand as follows.

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[1] Or factors. Throughout the present discussion it will be assumed for the sake of verbal economy that the characters in each instance depend upon the action of a *single* gametic factor. It makes no difference to the argument whether this is or is not true in a particular instance.

$$\text{P Generation}\quad \text{Black } ♂ \left\{\begin{matrix} bwNf\ .\ bwNf \\ \text{or} \\ bwNf\ .\ bwnf \end{matrix}\right\} \times \left\{\begin{matrix} BWnF\ .\ bWnf \\ \text{or} \\ BWnF\ .\ BWnf \\ \text{or} \\ BWnF\ .\ bwnf \\ \text{or} \\ BWnF\ .\ Bwnf \end{matrix}\right\} \text{White } ♀$$

F₁ Generation

♂ unknown

Faintly barred white ♀
(1) $bwNf\ .\ BWnF$
or
(2) $bwnf\ .\ BWnF$

Of these two alternative formulæ in F_1 the first is decidedly the more probable, since there is every reason to expect that (2) would be a pure white bird showing somatically no trace of barring.

In the F_2 generation got by mating the faintly barred white ♀ of F_1 to the black ♂ of the P generation the *females* were barred, indicating again that one of the gametes uniting to form these individuals must have borne both F and B, since no gamete from the sire could bear either of these factors.

It is evident that in interpreting this case we are forced to adopt either one or the other of two alternatives, both of which present novel points in comparison with the results of recent experiments regarding the inheritance of the barred pattern in crosses involving Barred Rocks, in which this pattern is well fixed. On the one hand we may conclude that the White Booted ♀ original parent carried the B factor in its gametes. This interpretation leads to the results worked out above, the novel point in which is that here there is no *repulsion* between B and F in gametogenesis (or *coupling* between B and f if one chooses that view) as is the case in Barred Plymouth Rocks of the present day. Here a non-barred ♂ mated with a female carrying barring (by hypothesis) produces *barred daughters*, where there should be produced (to accord with recent experiments on barring) barred sons and *non-barred* daughters. On the other hand it is possible to assume that the faint barring in the F_1 ♀ arose *de novo*, and that the White Booted ♀ parent did *not* carry the B factor. On this view it must be concluded that *this new character barring when it first appears behaves in an absolutely different way in inheritance from what it does later.* Either conclusion is sufficiently interesting, and stimulating to further research.

Of course a third assumption still is possible, namely that the barring of the Cuckoo Pekins is a different barring entirely from that of Barred Plymouth Rocks, and therefore behaves differently in inheritance. There is no evidence, however, on which to base such an assumption. All of the types of barring which do behave differently in inheritance from the Barred Rock type (*e. g.*, the Campine or the Pencilled Hamburgh barring) are somatically distinctly different from the Barred Rock type of barring. The barring of the Cuckoo Pekin, however, belongs to the same type somatically. This is shown in Fig. 1, which is a copy of a picture of a Cuckoo Pekin ♂ drawn by the well-known

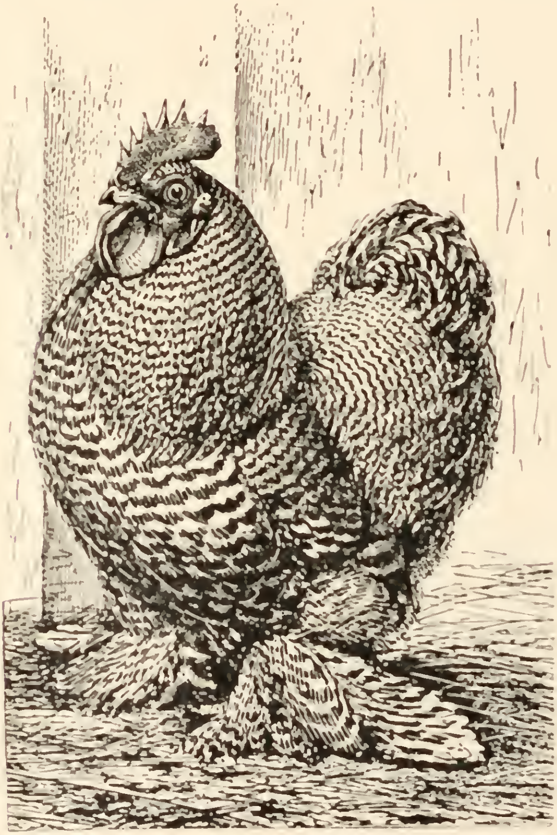

FIG. 1. A Cuckoo Pekin ♂, winner of first and special at Whitby, first and special at Hallam and Ecclesall, etc., etc., 1894. From Entwisle, *loc. cit.*, p. 20.

English poultry artist Ludlow, and published in a plate facing p. 20 of Entwisle's[1] book.

One of the most important and most baffling problems in genetics today is the analysis of "pattern" inheritance. There is definite and indubitable evidence that certain plumage patterns in poultry behave as clean-cut unit characters, dependent on gametic determiners or factors which act precisely like pigment factors for example. Yet the gametic and ontogenetic mechanism of pattern determination and development is most difficult even to imagine. There certainly is great need for further research in this field. Particularly it is important to see whether definite patterns may be formed *de novo* from crosses of birds which bear no trace of the determiners of the patterns gametically.

The case here under discussion illustrates the difficulties which attend the getting of really critical evidence on this matter for the barred color pattern. Merely to show that a black and white bird mated together give barred offspring will not suffice. This happens if one mates any bird carrying black pigment with a White Plymouth Rock, but it is merely (and obviously) because the White Rock carries the barred pattern factor as a cryptomere. To get crucial evidence one must use black and white breeds (*a*) in which there is no evidence of barred birds having been used in the crosses from which the breeds were originated; (*b*) in which there never occur barred "sports"; (*c*) in which barred varieties of the breed are unknown; and (*d*) in which the white of the white parent is a "recessive" and not a "dominant" white. These criteria at once exclude from experiments on synthesizing the barred pattern from black × white crosses, if such experiments are to be really critical, all Mediterranean breeds (so far at least as these breeds are known to the writer). Further the stock used must be given a thorough preliminary test in Barred Rock crosses to determine whether it does or does not carry the *B* factor. Experience indicates that it is difficult to settle this point if the white of the white race belongs to the "dominant white" category. There are, however, certain races of poultry which seem to fulfil the requirements for a crucial test of the fundamental question of the *de novo* formation of the barred pattern from a black × white cross. Experiments are now being carried on in this direction by the writer.

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[1] *Loc. cit.*

II. The Color and Pattern of Early Barred Plymouth Rocks.

The first Barred Plymouth Rock fowls to be entered under this name at a poultry show were exhibited in 1869 by D. A. Upham at Worcester, Mass.[1] They had first been bred some four or five years before that time. So far as I have been able to learn the earliest published picture of fowls of this new breed of fowls, which was in any degree an accurate or adequate representation of the actual birds, first appeared in the *American Agriculturist* in January, 1872.[2] The well-known poultry artist Mr. Franklane L. Sewell states[3] that he knows of no earlier picture than this and his experience in this field is extensive. Whether this picture is absolutely the first of the breed to appear is not essential, nor of any interest other than purely antiquarian. The biological interest of the picture lies first in the fact that it shows the appearance of birds of this breed very early in its history, and second in the fact that the drawing is well done, and may be taken with considerable confidence as an accurate representation of the appearance of the best of the Plymouth Rock fowls of that time. This wood-cut, which is here reproduced as Fig. 2, bore originally the initials, "E. F.," which were those of Edwin Forbes, a rather clever delineator of poultry, who worked in New York.

The picture was copied, or rather apparently printed from the same block, without credit for a prior appearance, and with the artist's initials erased, in the *Poultry World*[4] for July, 1872. It is from this publication that the picture has been chiefly known to poultry fanciers. Sewell[5] gives the *Poultry World* as the original place of publication.

[1] Robinson, J. H. "Principles and Practice of Poultry Culture." Boston, 1912, Ginn & Co., p. 399.

[2] Vol. 31.

[3] Sewell, F. L. "Color of Plumage of the Barred Plymouth Rock," *Reliable Poultry Journal*, Vol. XV., p. 520, July, 1908.

[4] Vol. I., p. 85. This journal which has long since ceased to exist, was published at Hartford, Conn.

[5] *Loc. cit.*

The drawing was made from actual birds, and is therefore not wholly imaginary or idealistic. The pair of birds figured belonged to a Mr. C. C. Corbett, of Norwich, Conn., and represented a high degree of excellence for the time.

The chief points of interest in this picture for the student of genetics are the relatively dark color of the birds and the indistinctness of the pattern. In both respects, of course, these birds stand in marked contrast to the Barred Rocks of the present day. While a generally barred effect is evident, the pigment obviously spreads over a great deal of the feather, encroaching on the areas which are white in a modern Barred Rock.

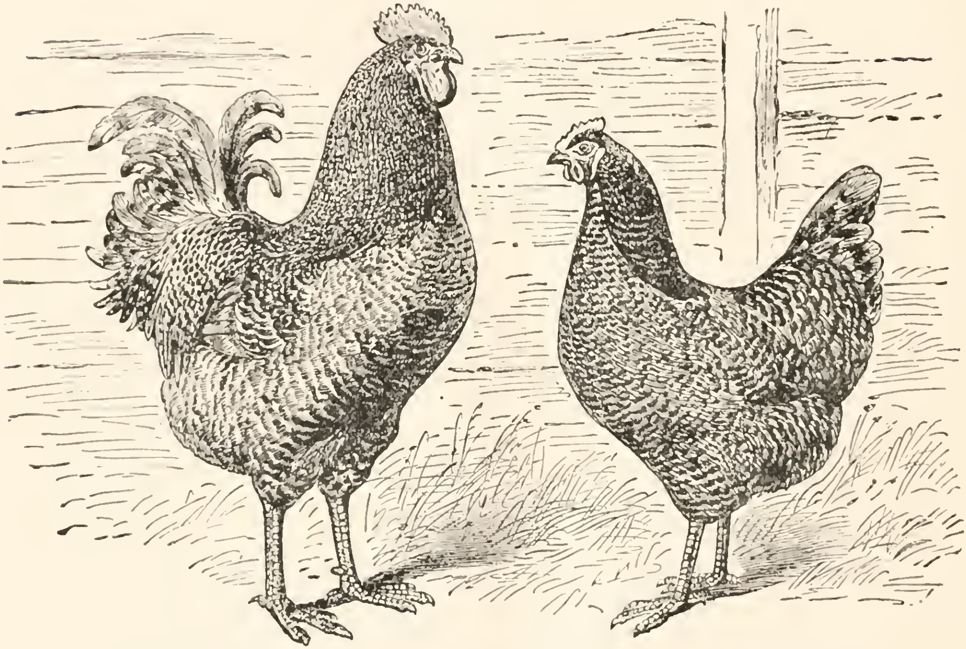

FIG. 2. Plymouth Rock fowls as they appeared in 1872. This is believed to be the earliest adequate picture of birds of this breed.

The fact to which I wish especially to call attention is that *these early Plymouth Rocks were evidently very similar indeed in color and color pattern to the F_1 birds obtained by crossing a modern Barred Rock ♂ with a ♀ belonging to some heavily pigmented breed, such as for example the Cornish Indian Game.* Allowing for differences between modern photographic technique and a wood-cut printed on poor paper, and for differences in shape of body, due to the game blood, the similarity between the birds in Fig. 2 and the barred ♂ and ♀ of the F_1 generation of the cross B. P. R. ♂ × C. I. G. ♀ published in Roux's Archiv[1] is striking. The male plumage is relatively dark in both cases as compared with that of the modern pure B. P. R. ♂. In the female there is also an excess of pigment, seen not only in the general color tone

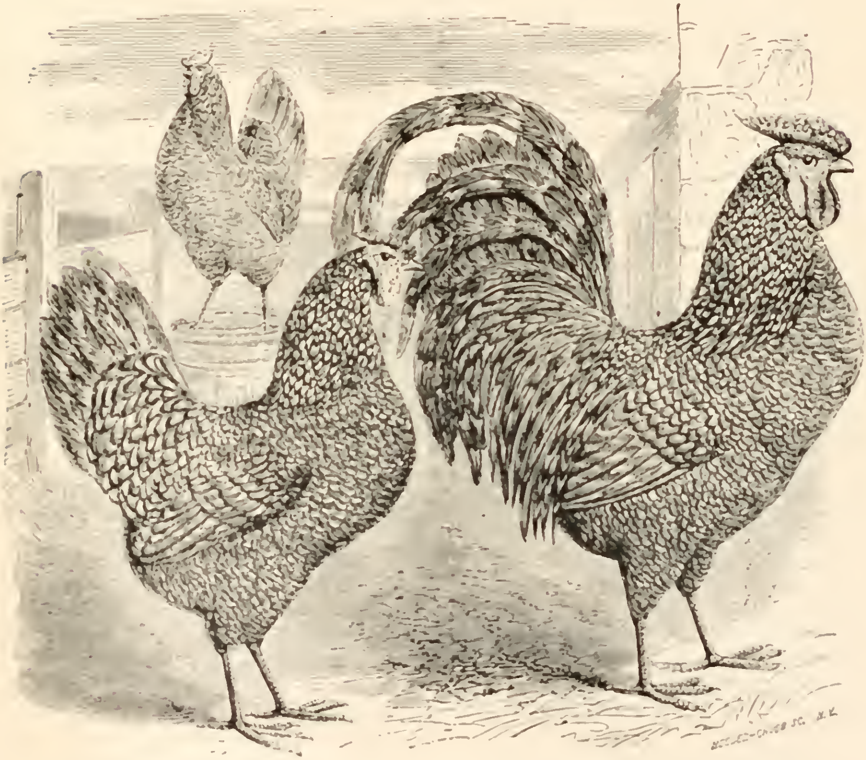

FIG. 3. Reproduction of a wood-cut of Dominiques, originally published in 1870.

of the bird, but in the apparent "smokiness" due to the encroachment of the black bars on to the white areas of the individual feather.

Now in the case of the F_1 birds it is clear what causes (or is at least associated with) the defective development or expression of the pattern. The pure B. P. R. ♂ of today is, so far as is known, always homozygous with reference to the barring factor, whatever that may be. He carries two "doses" of *B*. Somatically he is light in color with narrow clean cut bars. When by crossing a male is made carrying but one "dose" of *B* (*i. e.*, heterozygous in relation to the barring factor) the somatic pigmentation is markedly changed, and becomes like that of the earliest Barred Rocks known. This obviously suggests that in the early history of the breed the males were regularly heterozygous with reference to barring. If so they should have produced, with considerable regularity, non-barred (black) daughters. As a matter of fact this was probably the case. Up until 20 years ago, and even later in some localities, one would judge from various statements to be found in poultry journals, agricultural papers and the like, that it was not a particularly uncommon occurrence for a Barred Rock mating to throw some solid black chickens.

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[1] Pearl and Surface, *loc. cit.*

It is of interest to note that at the time of the original foundation of the Plymouth Rock breed the Dominiques, from which the barred pattern was derived, had the same type of pigmentation. This is shown in a contemporary wood-cut of the latter breed, reproduced in Fig. 3.

This picture of Dominique's was published in the *American Agriculturist*, Vol. 29, p. 13, 1870. It was drawn by Edwin Forbes from a pair of birds owned by Col. Henry Howland of Chicago. These birds were prize winners in their time. This cut, in a very much garbled form, was reproduced in the *Fanciers Journal* in 1876, from which source it has been copied by Sewell.[1]

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[1] *Loc. cit.*

COMPLETE DISCHARGE OF MITOCHONDRIA FROM THE SPERMATOZOÖN OF PERIPATUS.[1]

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The following account presents the unique case of a spermatozoön that loses all its mitochondria with the abstriction of the cytoplasm.

In my paper ('00) on the spermatogenesis of *Peripatus balfouri* the history of the germinal cycle was described from the spermatogonia through the maturation divisions, and that is now completed by a description of the spermiogenesis. In that account certain distinctive bodies were figured and discussed under the name of "yolk spherules." These were noted to occur only sparsely in the spermatogonia, but to become abundant during the growth period of the spermatocytes, and in all mitoses to lie outside of the spindles. Similar bodies were seen in the sheath cells of the testis, and I concluded these to be nurse cells, elaborators of the supposed yolk spherules, and that the spermatogonia received their "yolk spherules" from these nurse cells. At the time when that paper was sent to press Benda had not yet published his term mitochondria, and in conformity with the results of other investigators of that day I supposed true yolk to be formed within spermatocytes.

Now I am able to demonstrate that the bodies in question are not yolk spherules, rather chemically quite different from these, but are mitochondria according to their behavior and staining reactions. Indeed, it will probably be found that most bodies described as yolk spherules in spermatocytes are really mitochondria. My early account was therefore one of the first to describe mitochondria through successive cell generations of spermatogenesis. But that account was wrong in its inference that those of the germ cells proper are derived from those of the nurse cells; on the contrary, they occur independently in the two classes of cells, just as they do in the Sertoli cells and spermatocytes of mammals.

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[1] *Note by the Editor.* Professor Montgomery died while this paper was in press. He had therefore no opportunity to make any changes in the proof, and the paper is printed exactly as the manuscript left his hands.

The material consisted of testes, seminal vesicles, vasa deferentia, and oviducts, some fixed in strong Flemming's fluid diluted with an equal part of distilled water, and others preserved in corrosive sublimate-acetic; all were originally received from my friend, Dr. Purcell, of Cape Town. The mitochondria appear pale red after Hermann's safranine-gentian violet, and after the Ehrlich-Biondi-Heidenhain method; shades of gray or black after iron hæmatoxylin, according to degree of destaining; and after Benda's stain they are deep violet, while the chromatin is brownish and the centriole red—the typical reaction to this stain. There is only one other object known to me on which they are equally readily demonstrated, namely, the spermatocytes of *Ascaris*.

Fig. 1, Pl. I., exhibits the position of the mitochrondia at the end of the second maturation mitosis where, as after the first also, they lie at the distal poles (equatorial ends) of the daughter cells. Until their later fusion takes place they are chiefly peripheral, next the cell wall, spherical or slightly elongate, and in the form of hollow vesicles. In the earliest spermatids they always form a layer at the distal pole, but sooner or later move forward, along the cell membrane, so as to take a position on the side of the nucleus (Figs. 2–16); at the same time the sphere (*s*) always advances from its original position and the cytoplasm comes to make a lobe around the nucleus and entirely in front of the centriole (*c*). These movements do not occur synchronously on cells of the same stage, there is much variation in the process, yet the end result is the same in all. By reason of the mitochondria remaining generally in a single layer they may be readily counted, and their number is found to differ in different spermatids, which shows their mass cannot be accurately quartered by the maturation divisions. In Figs. 4, 6, 11–13, all of each cell are drawn with care, and their numbers here are respectively: 33, 45, 68, 64, 49. Their volumes also differ considerably, as the figures show. They do not blacken with osmic acid.

In the nucleus the chromosomes are at first peripheral and quite distinguishable (Fig. 2), then coalesce to produce a hollow chromatin sphere enclosing clear karyolymph (Fig. 3). Next the nucleus lengthens, and distally its chromatin border becomes very thin (Figs. 4, 5, 7, 8); but it could not be determined that at this thin region nuclear sap passes out of the nucleus in the way I have described (1911) for *Euschistus*. This thin area of the nuclear wall later becomes as thick as the remainder (Figs. 9, 10). Then the proximal end of the nucleus becomes pointed (Fig. 10). With its later great elongation (Figs. 11–17, and Plate II.) the nucleus changes its appearance, due apparently to its interior becoming more chromatic, so that on surface views it appears nearly homogeneous throughout; it continues the same affinity for basic stains, and from the stage of Fig. 11 onwards I have drawn it brown and not deep black simply in order to represent the mitochondria more distinctly. Yet cross sections show that even in the mature sperm the chromatin makes a hollow cylinder and not a solid rod.

Now to return to the mitochondria, to describe their particularly notable phenomenon. After all of them have moved forward from the distal pole, carried probably by currents in the lobe of cytoplasm, they fuse together to produce a true Nebenkern or chondriosome (Meves, '00). Figs. 11–16 show them becoming agglomerated, and in Fig. 17 they are seen to be fusing to produce compound vesicles. Fig. 18, Pl. II., exhibits them on the side of the nucleus, with fusion far advanced, and Fig. 19, their consolidation into a chondriosome. As the process of fusion advances they stain more deeply, so that the chondriosomes when completed appear densely chromatic (Figs. 20–22). Sometimes a few mitochondria remain isolated without joining with the others, sometimes all fuse together (Figs. 18–21). Simultaneously the cytoplasmic lobe moves forward along the nucleus, or, probably more correctly, the nucleus moves backward through it; and in its substance appear denser strands and minute granules (Figs. 18, 19) which may be degeneration products comparable with the "tingible granules" of mammals. Consequently, each nearly mature sperm of the seminal vesicle (Figs. 20, 21), as all of the vas deferens (Fig. 22), carries near the anterior end of the nucleus a cytoplasmic lobe with a densely staining chondriosome; there appears to be no cytoplasm at all in the region of the centriole and the flagellum. Were the history of the spermatozoa unknown beyond their conditions in the vasa deferentia, there would be no evidence of the fate of the cytoplasmic lobes and chondriosomes. But fortunately I have numerous preparations of oviducts from female individuals, all crowded with spermatozoa, and in these all the spermatozoa lack entirely the cytoplasmic lobes and chondriosomes (Figs. 23, 24); in not a single case was a cytoplasmic lobe observed upon a spermatozoön when within an oviduct.

Peripatus, accordingly, has for us more than a phylogenetic interest, it has a high cytological importance. The spermatozoön during its development casts off its cytoplasm, and evidently all of it. But this abstriction of the cytoplasm, or a portion of it, is now known to be a quite general phenomenon in animals, and only amphibians and certain insects appear to furnish exceptions to it. Much more important is that all the mitochondrial substance, in the form of a compact chondriosome, is cast away with it. Further, I had previously described the spermatozoön as possessing a lance or perforatorium, staining differently from the nucleus. Now I can demonstrate that this supposed perforatorium stains differently only on account of its excessive tenuity, that it is only the narrowed proximal end of the nucleus, and that it has no connection with the sphere. We have seen that the sphere arises just behind the nucleus (Figs. 2, 3), and moves forward into the cytoplasmic lobe (Figs. 5, 7, 8, 10, 12, 14, 16–19). When the chondriosome is fully developed the sphere lies still in the cytoplasmic lobe, separated from the nucleus (Fig. 20), and no evidence was observed that it moves along the latter to constitute a perforatorium. Therefore it is certain that the sphere as well as the chondriosome becomes thrown off with the cytoplasm.

The history of the centriole was not followed in detail. In the telophase of the secondary spermatocytes a minute centriole is present at each pole (Fig. 1). At the next stage when it was noticed (Fig. 3) it appeared as a much more voluminous body at the distal pole of the nucleus, and it retains this position thereafter. Later it becomes discoidal with indication of subdivision into two parts (Figs. 4–10), and afterwards lengthened in the axis of the spermatid (Figs. 11–19), reaching its maximum size at the stage of Figs. 13, 14. In the mature and nearly mature spermatozoa it makes a slender rod, joining nucleus and flagellum, and then is seen to have decreased in volume (Figs. 21–24). The flagellum connected with it is a delicate, flattened thread, evidently without spiral membrane or cytoplasmic sheath; in the figures only its proximal portion is shown. In some cases there appeared to be a spiral skeleton around the nucleus, such as Koltzoff (1908) has recently described in other species; but examination proved that in *Peripatus* this is occasioned simply by chance wrapping of a flagellum around the nucleus.

In the mature egg and in cleavage stages no structures were found in any way resembling the mitochondria of the sperm cells.

Meves (1908, and later papers) draws the conclusion that mitochondria are important hereditable elements, directing cytoplasmic activities as the chromosomes direct those of the nucleus, self-perpetuating bodies differentiating during ontogeny into most of the fibrillar structures of the body. Without entering into the rapidly growing literature now, we will be content with the statement that a considerable number of investigators corroborate these views, and that they have been especially elaborated by Giglio-Tos and Granata ('08). This hypothesis more than any other has directed attention to these bodies. Yet they are far less conservative and regular than the chromosomes in number, form and behavior and there is evidence that occasionally some of them are eliminated during spermiogenesis. Thus Fauré-Fremiet has distinguished four types of them: (1) Those that undergo changes of position without profound morphological changes, as in mammalian spermiogenesis. (2) Those that at the same time undergo great structural changes, as in insect spermatids. (3) Those of which only a part change into the chondriosome or Nebenkern of the spermatid, while others degenerate, as in spermatogenesis of certain gastropods. And (4) those that transform wholly or partly into deutoplasmic bodies, some cases of oögenesis. The fourth of these classes cannot be said to be definitely established, but there can be little doubt about the evidence for the third. Thus, besides the instance in gastropod spermatogenesis studied by Fauré-

Fremiet, Retzius noted in mammals a "considerable reduction in their substance as they enter into the formation of the spiral thread." Jordan ('11) found that in spermatids of the opossum a considerable number of mitochondria are cast off with the cytoplasm; and while Duesberg ('10) maintains that in the guinea pig all take part in forming the spiral thread of the spermatozoön, yet he figures granules with similar staining reactions in the dehiscent cytoplasmic lobe. The parallel does not seem yet to have been made, yet may not the "tingible corpuscles" of mammalian spermatids be metamorphosed mitochondria, ones that have nothing to do with the spiral threads? And now we are able to adduce the positive case of *Peripatus*, in which all the mitochondria become removed from the spermatozoön.

In view of these facts it seems to me we should be very cautious in attributing to the mitochondria a rôle in cellular activity at all equal to that of the chromosomes. No spermatozoön ever discards chromosomes, but that of *Peripatus* throws off all its mitochondria.

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Explanation of Plates.

All figures were drawn with the cameral lucida at the level of the base of the microscope, Figs. 13, 20 with Zeiss apochr. 1.5 mm., oc. XII., the others with Zeiss achr. 1/12 mm., oc. XII. Figs. 1–3, 5, 7–12, 14–19 from a seminal vesicle, Flemming's fluid, iron hæmatoxylin; Figs. 4, 6, 21 from a seminal vesicle, corrosive sublimate-acetic, iron hæmatoxylin; Figs. 13, 20 from a seminal vesicle, Flemming's fluid, Benda's stain; Fig. 22, from a vas deferens, Flemming's fluid, safranine-gentian violet; Fig. 23, from an oviduct, treated like the last; Fig. 24, from an oviduct, corrosive sublimate-acetic, iron hæmatoxylin.

c, centriole.

s, sphere.

Plate I.

Fig. 1. Telophase of second maturation mitosis.

Figs. 2–17. Successive stages of spermiogenesis from the seminal vesicle. Fig. 6 is an apical view of the stage of Fig. 5.

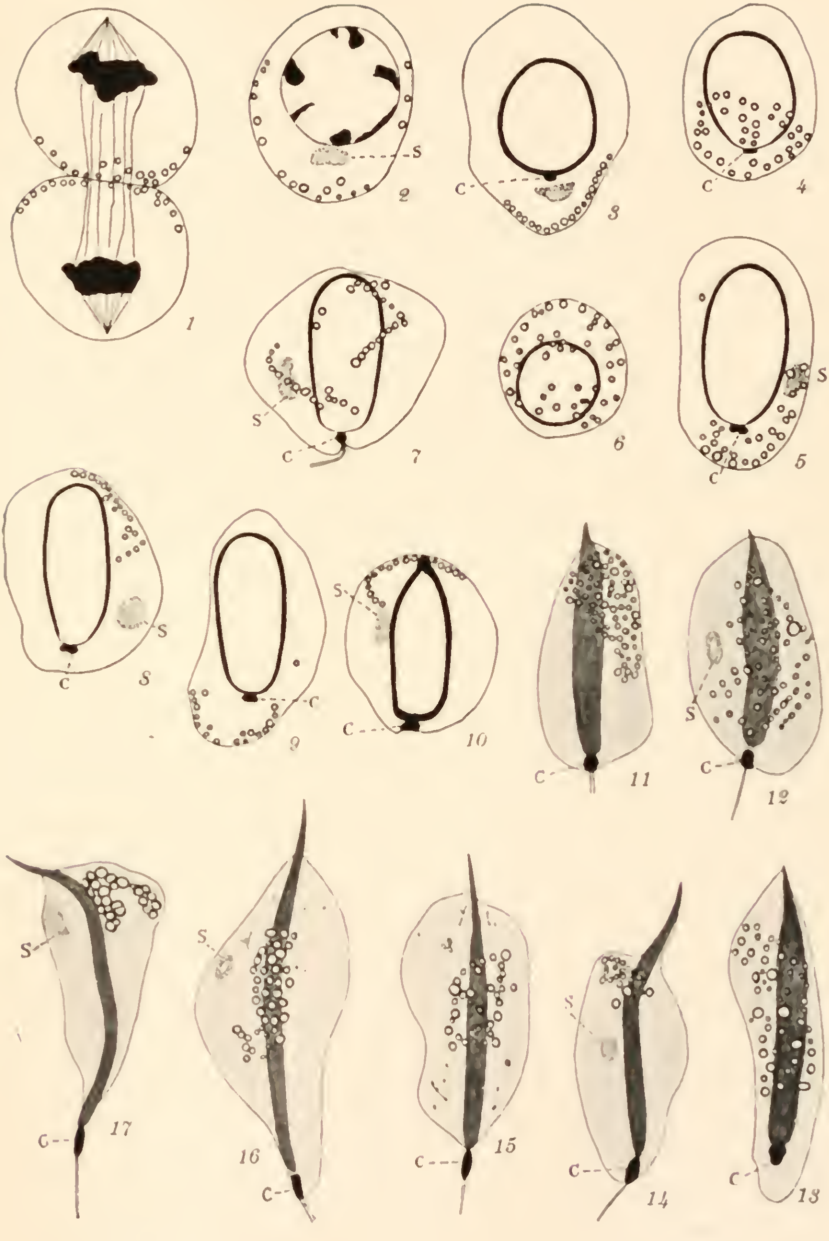
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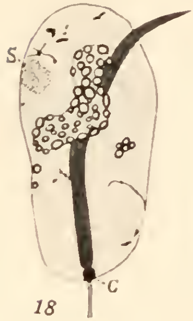
PLATE II.

FIGS. 18–21. Later stages of spermiogenesis from seminal vesicle. In Fig. 20 only the cytoplasmic lobe and the proximal portion of the head are shown.

FIG. 22. Spermatozoön from vas deferens.

FIGS. 23, 24. Spermatozoa from oviduct.



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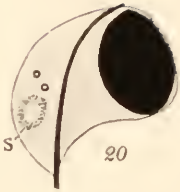

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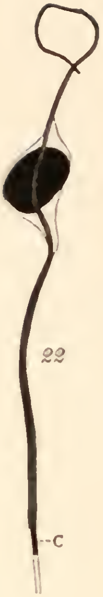

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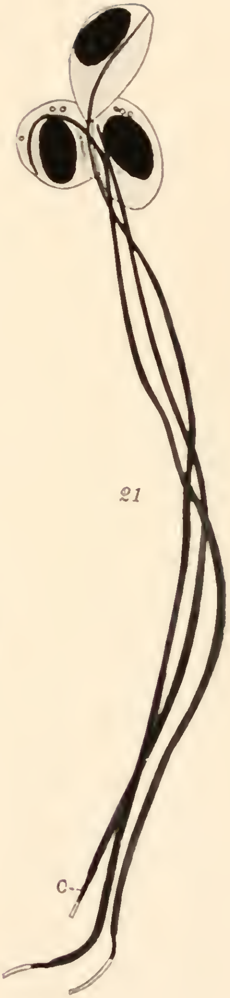

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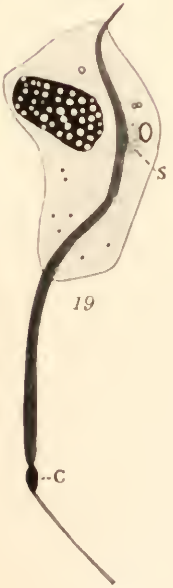

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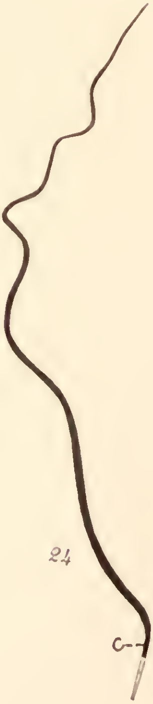

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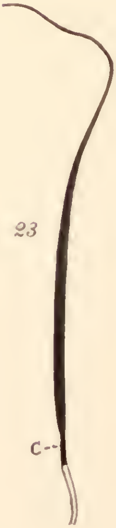

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T. H. MONTGOMERY.

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BIOLOGICAL BULLETIN

A PRELIMINARY ACCOUNT OF THE DEVELOPMENT OF THE APYRENE SPERMATOZOA IN *STROMBUS* AND OF THE NURSE-CELLS IN *LITTORINA*

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The dimorphic spermatozoa in *Strombus* were first described by Brock in 1887 in the case of *S. Lentiginosus.* While accurate enough in general, his account is lacking in certain interesting details and he also made no attempt to trace the developmental stages of either kind of spermatozoa Both his description and his figures, however, are sufficient to show how marked and striking is the dimorphism existing here and it is surprising that this has not been made the subject of further investigation by more recent workers.

My attention was attracted to *Strombus* in May, 1911, while at the temporary laboratory established by The Carnegie Institution of Washington at Port Royal, Jamaica, W. I. There I had the opportunity of observing the living spermatozoa and also of securing a lot of material for further study. The species studied was *S. bituberculatus.* My thanks are due to Dr. H. A. Pilsbry of The Academy of Natural Sciences of Philadelphia, who kindly identified it for me.

Adopting the terminology suggested by Waldeyer and used first by Meves ('03), the two kinds of spermatozoa found in *Strombus* are the eupyrene, *i. e.*, those that function in the ordinary way, and the apyrene whose function is unknown and in whose adult structure there is no active nuclear material. The eupyrene spermatozoa do not present any striking differences from those found in other forms which have the same sexual dimorphism, *Paludina* for example, but they lack the tenuous perforatorium and the very long tail-piece of the spermatozoa found in forms like *Littorina* and *Fasciolaria* (Fig. 3). The apyrene spermatozoa, on the other hand, bear very little resemblance to those described in other forms and to the eupyrene spermatozoa they bear absolutely none at all. They are very much larger than the latter and are outnumbered by them, according to Brock's estimate, about 1 to 500; the correct numerical relation existing between them in *S. bituberculatus* has not been ascertained but it is probably the same as in *S. lentiginosus*. In length the apyrene spermatozoa average somewhat over 90 micra.

The adult apyrene spermatozoön is composed of a central spindle-shaped cell body, which is long and narrow and slightly flattened dorso-ventrally, and two undulating membranes which pass down either side of the cell-body (Fig. 2). At the anterior end of the spermatozoön the membranes round out sharply to their maximum width while posteriorly they narrow more gradually and finally end in a short sharply pointed tail-piece. The interior of the cell-body is filled with a number of large polygonal bodies composed of an albumen, probably a nutritive material. These bodies are more or less regular in shape and position but they decrease in size at either end of the cell.

The living spermatozoa, as they leave the sperm-ducts, do not show any violent movements; at first long slow contraction waves pass alternately down the two membranes in a postero-anterior direction, propelling the spermatozoön in the opposite direction, that is, the posterior end is directed forward.[1] Occasionally a spermatozoön is seen moving with its anterior end directed forward. The movement of the spermatozoön is comparatively slow and is not long continued as it soon attaches itself by means of its tail-piece to the glass slide or other object upon which it is being observed. As soon as this occurs the contraction waves pass down both the membranes simultaneously and they become much shorter and faster. With the spermatozoön attached in this way, the membranes may continue to be active for an hour or two. It very frequently happens, however,

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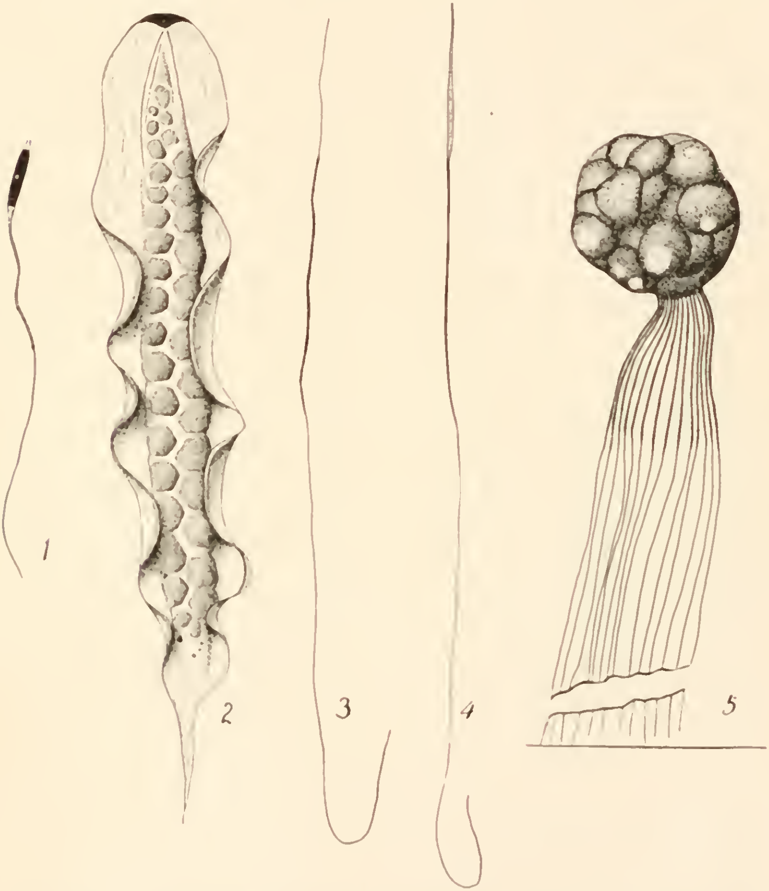
[1] In *Paludina* that end of the spermatozoön which contains the remains of the nucleus, *i. e.*, the head, has been designated as the anterior end and this is the end which is directed forward in movement; it is also the end toward which the axial fibers have grown. In *Strombus*, on the other hand, there is no nuclear head in the spermatozoön and therefore, following the precedent established above, I have designated as anterior that end toward which the axial fibers have grown. It happens as a rule that in movement this end is directed backward. It was thought better to orient the spermatozoön morphologically rather than by the direction of movement. To be correct, the orientation should be reversed in both cases as the end of the eupyrene spermatozoön toward which the axial fiber has grown is the posterior one.

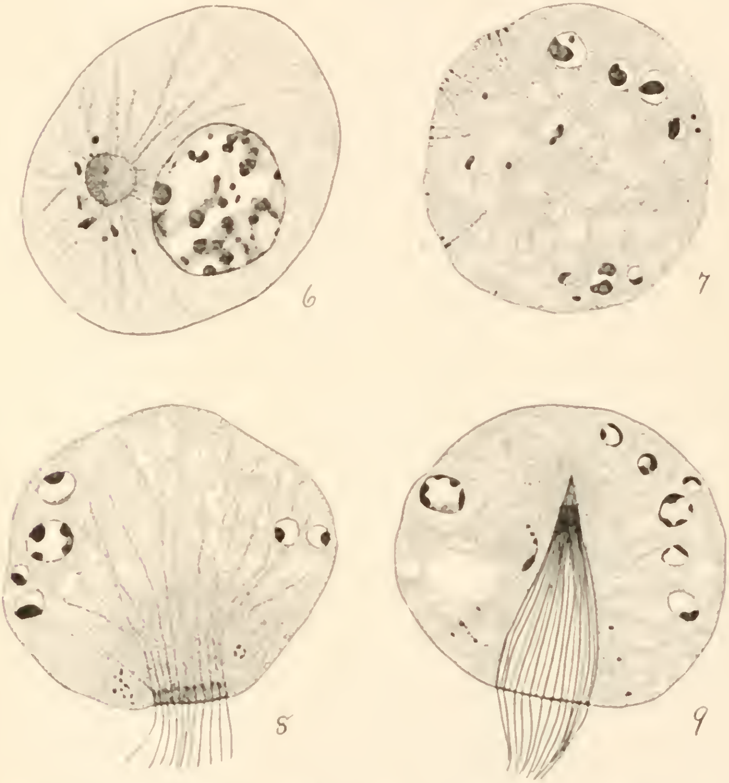


FIGS. 1 to 5. Initial magnification of 1,850 diameters, reduced one third. Fig. 1, eupyrene spermatozoön of *Strombus bituberculatus*. Fig. 2, apyrene spermatozoön of the same form. Fig. 3, spermatozoön of *Littorina nebulosa* showing the long, thin perforatorium. Fig. 4, spermatozoön of *L. angulifera* showing the perforatorium swollen after being in sea-water for some time. Fig. 5, nurse-cell of *L. nebulosa* with attached spermatozoa; drawing made from a living cell.

that the tail-piece breaks off and the spermatozoön swims away with a much more rapid movement than it had at first. When the tail-piece begins to break off it can be seen to be composed of a number of fused flagella; sometimes as the spermatozoön moves away one or more of the flagella may be seen still adhering to it. This explains the statement of Brock to the effect that a tuft of flagella, which is invisible at first, is to be seen after the spermatozoön has been swimming about for a while. It very frequently happens that long before the undulations of the membranes have ceased, the spermatozoön flattens out and the albuminous bodies break down, leaving in their place a brownish, semi-fluid substance in which, however, may still be seen the outlines of those bodies.

As in *Paludina*, the apyrene spermatocytes of *Strombus* are easily recognized. They are large pear-shaped cells provided with a nucleus of regular pattern and a large centrosome about which may be seen an inner clear court and an outer dark court. Until a very late stage in their growth period they retain a connection with the cyst-wall of the testis by means of a short stalk; later they lose this attachment and become spherical. By this time the chromatin has begun to form in lumps beneath the nuclear membrane and from a large number of centrioles lying at the periphery of the centrosome strong radiations may be seen to pass out in all directions (Fig. 6). In the outer court, but away from the nucleus, lies a mass of mitochondria. A division of the nucleus and cell never follows; instead, the nuclear wall breaks down and the centrosome with its radiations disappears. A little later the chromatic masses are seen scattered through the cell while the centrioles have moved to the periphery of one half of the cell where they are easily recognized by their radiations (Fig. 7). The cell now begins to develop directly into the spermatozoön.

The chromatin, as such, takes no further active part in the development of the spermatozoön; the fragments very soon begin to become vesiculated and to degenerate. The centrioles mass together at a point just beneath the cell membrane where they divide (Fig. 8). One half of the number of daughter or secondary centrioles remain attached to the cell-membrane and from them grow out flagella which ultimately fuse to form the tail-piece. The others move across the cell forming a bundle of axial fibers. At its base the bundle is round but it becomes more and more flatly oval as the fibers move across the cell (Fig. 9).



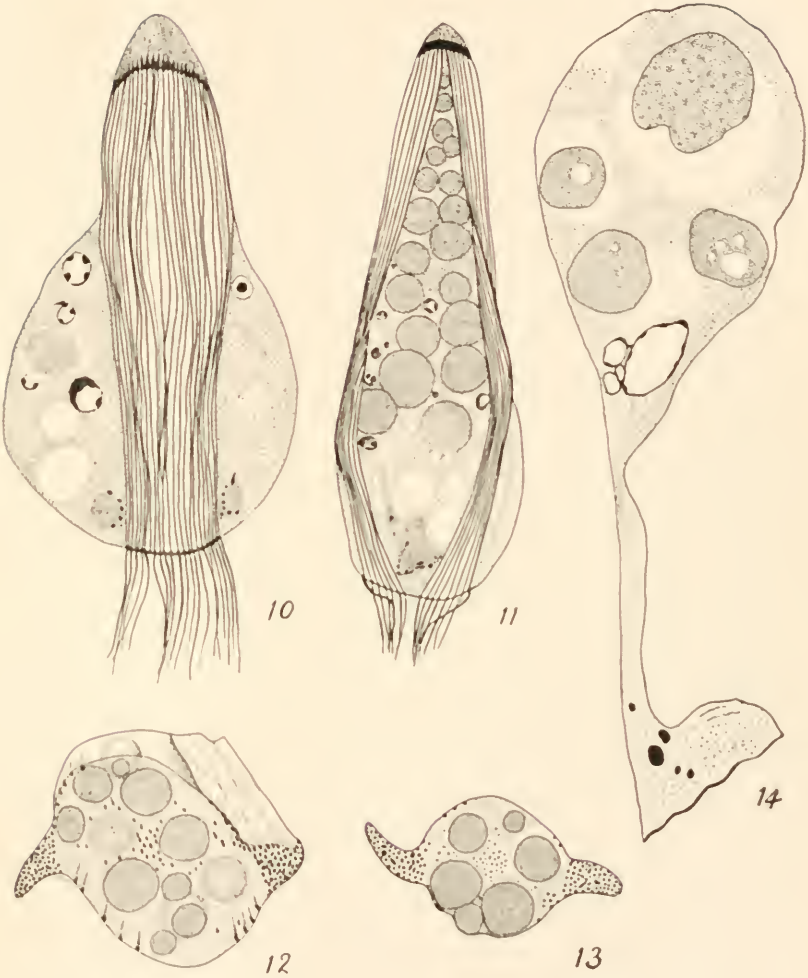
FIGS. 6 to 9. Initial magnification of 3,450 diameters, reduced one third. Various stages in the early development of the apyrene spermatozoa of *Strombus bituberculatus*. Fig. 6 is a spermatocytic stage just before the breaking down of the nucleus and the disappearance of the centrosome. Fig. 7 is a stage following the breaking down of the nucleus; the centrioles figured represent only a portion of the total number in the cell. Fig. 8 shows the beginning of the bundle of axial fibers. Fig. 9 shows the fibers grown partly across the cell; the view is taken at an angle of 90 degrees from that of Figs. 8 and 10.

The mitochondria may be seen to form the inner margin of a ring of differentiated cytoplasm which surrounds the base of the bundle. They persist here until the spermatozoön has reached its adult form (Figs. 8 and 10).

When the bundle of axial fibers, by its growth, has increased the length of the cell by about one third, it splits and the halves begin to move to either side of the cell (Figs. 10 and 11). This movement is caused by a twisting of the secondary bundles, due probably to the unequal growth of the fibers. This process continues until they push out the cell membrane on either side to form the undulating membranes (Figs. 12 and 13). Fig. 12 is an oblique section through the middle of a spermatozoön which has not quite matured; later the membranes become flatter and wider as shown in Fig. 13, which is a more anterior section of a still older spermatozoön. As is indicated in Fig. 12, the continued growth of the fibers without a compensating increase in the length of the cell causes several even folds to occur throughout the length of the membranes. Some of the axial fibers do not participate in the formation of the membrane. As the bundles begin to evaginate the cell membrane, a few of the fibers begin to migrate and finally come to lie longitudinally across the cell and just beneath its membrane (Figs. 12 and 13).

The albuminous bodies are formed in the same way as Kuschakewitsch ('11) has briefly described in *Vermetus gigas*. Large vacuoles appear in the cytoplasm, first in the anterior portion of the cell, and these gradually become filled with an albuminous substance. When such a vacuole has been almost filled but before a membrane is formed, narrow strands may be seen connecting the albumen with the surrounding cytoplasm (Fig. 11). The cytoplasm which is not displaced by the formation of these bodies becomes fibrillar, the greater part of it forming a core down the center of the spermatozoön (Figs. 12 and 13).

As the spermatozoön develops, the vesiculated nuclear fragments which were scattered throughout the cell continue to degenerate. They gradually become more condensed and darkly staining and undergo further fragmentation. They may dissolve *in situ*, but they may also go to form the many small granules that lie in amongst the fibers composing the undulating membranes (Fig. 13). These granules are probably mitochondria and while at present it cannot be definitely asserted that they are thus of a direct nuclear origin, there is considerable evidence in favor of this view. The mitochondria which originally sur-



FIGS. 10 to 14. Initial magnification of 3,450 diameters, reduced one third. Figs. 10 to 13, various stages in the later development of the apyrene spermatozoa of *Strombus bituberculatus*. Fig. 10, bundle of axial fibers beginning to split; a few albuminous bodies, not figured, have been formed in the anterior portion of the cell. Fig. 11 shows the completed splitting of the bundle of axial fibers; the cell is not cut through its greatest breadth. Figs. 12 and 13 are sections through two nearly adult spermatozoa. Fig. 14 is a nurse-cell of *Littorina nebulosa* still attached to the wall of the testis; the stalk was about to be severed just below the degenerating nucleus.

rounded the centrosome remain at the posterior end of the spermatozoön.

The species of *Littorina* in which have been found a free nurse-cell to which the spermatozoa are attached are *L. angulifera*, *L. nebulosa* and *L. rudis*. The first two species were studied at Port Royal, Jamaica, along with *Strombus bituberculatus*, and they too were identified by Dr. Pilsbry.

If the sperm-ducts of *L. nebulosa* or *L. rudis* are ruptured, the contents, when diluted with sea-water, will appear under the microscope as a great number of spheres to each of which is attached a tuft of spermatozoa. The spheres are nurse-cells composed of vacuolated yolk bodies and a degenerate nucleus (Fig. 5). The nurse-cells of *L. angulifera* differ from those of the other two species in that here the yolk bodies are not vacuolated and they partly enclose a long thick cytoplasmic rod to one end of which are attached the spermatozoa. In all three species only the perforatoria and possibly the tips of the heads of the spermatozoa are inserted into the cell.

The first movement to be seen is a rhythmical and uniform beating of the tuft of spermatozoa which sends the nurse-cell rapidly forward. Later the spermatozoa beat independently and the tuft spreads. Very frequently the spermatozoa of one nurse-cell become entangled with those of another; in that event the nurse-cells are drawn together and held by an agglutinous substance forming the pabulum in the cell into which the spermatozoa are inserted. In this way a great many nurse-cells are drawn together and from such a mass the spermatozoa may later be seen protruding on all sides and beating regularly like cilia. This beating of the spermatozoa will continue for several hours.

In case a nurse-cell has not become entangled with others, the spermatozoa soon free themselves; their heads become further and further separated from the nurse-cell until the attachment is completely lost. A sperm thus freed is seen to have a long, thin perforatorium behind which is the head; the latter passes almost imperceptibly into a very long tail (Fig. 3). After the spermatozoön has been swimming about in the water for a time the perforatorium becomes swollen; this is what has usually been figured as the sperm head. The whole process can be seen to better advantage in *Fasciolaria*.

The nurse-cells develop from large cells which are attached to the walls of the testis by a long stalk. These cells closely resemble the apyrene spermatocytes of *Strombus* except that they lack the pronounced centrosome of the latter. The only indication of such a structure in the case of *L. nebulosa* is a series of fibers running up one side of the cell. This later disappears and probably forms the portion of the cell to which the spermatozoa are attached (Fig. 14). In *L. angulifera*, in the early stages of the nurse-cell, there is a darkly staining body lying in the cytoplasm which grows to form the rod spoken of above; this also may be of a centrosomal origin. The formation of the yolk bodies is much the same as that of the bodies described in *Strombus*. They differ, however, in that here they reach a much larger size and then fragment into two or more parts. Coincident with this cytoplasmic differentiation the nucleus undergoes partial degeneration; it simply becomes more and more vacuolated but never completely disappears.

Before the nurse-cell has reached its full development it loses its connection with the wall and moves into the lumen of the testis. Here the spermatozoa become attached. The nurse-cells function as such in the sperm-ducts. Sections of *L. rudis*, made from a specimen killed in February, showed the nurse-cells in the sperm-ducts to be in a more or less depleted condition.

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CERTAIN MEANS BY WHICH STARFISH EGGS NATURALLY RESISTANT TO FERTILIZATION MAY BE RENDERED NORMAL AND THE PHYSIOLOGICAL CONDITIONS OF THIS ACTION.

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Last summer at Woods Hole, while investigating the influence of ether and other anæsthetics in protecting the unfertilized eggs of starfish against the cytolytic action of pure isotonic sodium chloride solution,[1] I observed that toward the end of the breeding season the eggs often proved abnormally resistant to this solution, withstanding in some instances exposure of more than three hours to 0.55*m* NaCl without losing the power of development on fertilization; in normal eggs this solution typically causes complete cytolysis and coagulation of the protoplasm in two hours or less. Other characteristic abnormalities of behavior were found to be associated with this abnormal resistance to salt solutions. Usually a large proportion of such eggs failed to undergo maturation in sea-water, and of those which matured only a small proportion developed to a free swimming stage on fertilization, and the resulting larvæ were largely abnormal. Also the mature eggs, if left unfertilized in sea-water, frequently failed to die and break down within the usual time, but remained clear and apparently normal in appearance for an unusually prolonged period. It is well known that unfertilized mature starfish eggs undergo spontaneously a characteristic cytolytic alteration, accompanied by a darkening or coagulation of the protoplasm, which is typically complete within twelve to fifteen hours after deposition;[2] in contrast to this behavior a considerable proportion of the eggs under consideration often remained clear and uncoagulated in sea-water for twenty-four and in some cases for forty-eight hours. These several peculiarities, (1) failure of maturation in a large proportion of eggs, (2) failure of those eggs which did maturate to develop after fertilization, (3) marked delay in the typical post-maturational cytolysis of unfertilized eggs, and (4) unusual resistance to the cytolytic action of salt solutions were found in more or less constant association with one another in numerous lots of eggs. All of these peculiarities are evidence of a certain inertia or resistance to change in the egg-system; such eggs fail to react, or react slowly and imperfectly to conditions which call forth a definite and regular response in normal eggs. The fact of their concurrence, as well as their general nature, suggests that some single structural or metabolic abnormality, whose general effect is to lower reaction-velocities in the egg-system, is responsible for all of these peculiarities of behavior.

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[1] *American Journal of Physiology*, 1912, Vol. 30, p. 1.

[2] Cf. J. Loeb, *Archiv für die gesammte Physiologie*, 1902, Vol. 93, p. 59.

The further incidental observation was made in several instances that such eggs after treatment for two or three hours with sodium chloride solution containing a little ether or chloral hydrate recovered to a considerable degree the power of developing to a normal larval stage on fertilization; *i. e.*, eggs so treated yielded more numerous and more active larvæ than the untreated eggs of the same lot, fertilized at the same time. Pure sodium chloride solution showed a similar though less marked action. The surprising result thus appeared that treatment with solutions which are markedly injurious to normal eggs may bring a certain proportion of these abnormal or "over-ripe" eggs into a condition—as regards power of development on fertilization—closely approaching the normal.

The following record will illustrate the above-described conditions in detail.

June 29, 1911. The eggs were removed from a considerable number of starfish at 10.00 A.M. A large proportion of these mixed eggs remained permanently immature in sea-water (with intact germinal vesicles), but about half underwent apparently normal maturation. On fertilization (at 3:30 P.M.) very few of the eggs—a fraction of one per cent.—formed blastulæ, most died in early cleavage stages, but many failed to cleave or even to form fertilization-membranes. The unfertilized mature eggs after twenty-four hours in sea-water remained for the most part clear and translucent with no sign of coagulation; a good many, however, showed apparently normal coagulation, while others showed an intermediate condition. After forty-eight hours many unfertilized mature eggs were still uncoagulated.

About two hours after removal from the animals the unfertilized eggs were placed in the following solutions: (1) pure 0.55*m* NaCl, and (2–10) 0.55*m* NaCl containing anæsthetics as follows: (2–4) ether, 0.6, 0.45, and 0.3 volumes per cent., (5–7) chloral hydrate, 0.6, 0.4 and 0.2 per cent., (8–9) chloroform, one sixth and one tenth saturated, and (10) ethyl alcohol, 5 volumes per cent. After three hours and fifteen minutes in these solutions the eggs were transferred to normal sea-water and washed free of the anæsthetics by two changes of sea-water; spermatozoa were then added. At the same time spermatozoa were added to the untreated eggs which had remained in sea-water. The results were as follows: of the control eggs, fertilized in sea-water without treatment, almost all died before reaching the blastula stage; only a few feeble blastulæ (a fraction of 1 per cent.) were found on careful search; many eggs failed to cleave or even to form membranes. The eggs exposed to pure 0.55*m* NaCl also formed few blastulæ, but these were relatively somewhat more numerous as well as more active than in the control; the eggs treated with 0.55*m* NaCl containing 0.45 and 0.3 vol. per cent. ether, especially the latter, showed a more decided improvement over the control, though the proportion of blastulæ was still small. The eggs from the other solutions showed no improvement.

A similar result was observed in a second series of experiments with eggs which showed similar peculiarities. Eggs treated for three hours with 0.55*m* NaCl containing 0.3 vol. per cent. ether gave about 5 per cent. of blastulæ, while of the control untreated eggs less than one per cent. reached this stage. Eggs similarly treated with solutions containing a higher proportion of ether (0.75, 0.6, and 0.45 vol. per cent.) showed no improvement over the control. In another series eggs exposed for 3 h. 45 m. to 0.55*m* NaCl containing 0.1 per cent. chloral hydrate gave considerably more blastulæ than the control eggs.

It is to be noted that the improvement in the developmental power of these abnormal or resistant eggs was produced only by the pure salt solution or by solutions with a *low* concentration of anæsthetic. The concentration of ether most favorable for retarding the cytolytic action of 0.55*m* NaCl is considerably higher—from 0.5 to 0.6 vol. per cent.[1] The present effect, however, is not due to a simple prevention of cytolysis; the improvement over eggs left in sea-water, none of which undergo cytolysis within the time of exposure, cannot thus be explained. The effect is different from a simply protective action; and since it seemed to be favored by weak solutions of ether, the experiment was tried of exposing a batch of similarly abnormal eggs to sea-water containing 0.3 vol. per cent. ether. After three hours the eggs were returned to normal sea-water and fertilized. Next day it was found that the great majority of mature eggs had formed active and vigorous blastulæ and gastrulæ, while of the untreated control eggs left in sea-water and fertilized at the same time as the others less than one per cent. formed larvæ and these were feeble and abnormal. It thus appeared that the abnormal condition which renders the egg incapable of responding fully to the fertilizing action of the spermatozoön might be removed by treatment with sea-water containing ether in certain concentrations, which are considerably lower than those required for typical anaesthetic or protective action.

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[1] Cf. R. S. Lillie, *loc. cit.*, p. 6.

What are the conditions of this effect? In a recent paper[1] I have presented evidence indicating that the protective and anæsthetic actions exerted by ether and other lipoid-solvents in certain concentrations are due primarily to a *modification of the plasma membranes* of the cells or irritable elements, of such a kind as to render these membranes more resistant toward agencies that under the usual conditions rapidly increase their permeability; cytolysis and stimulation, both of which depend on such increase of permeability, are hence checked or prevented. Decrease in the readiness with which the permeability is increased thus involves for an irritable tissue decreased irritability; this effect is produced by various salts, *e. g.*, of magnesium, and by ether and other lipoid-solvent anæsthetics in certain (not too high) concentrations. In lower concentrations it has been observed that ether and other lipoid-solvents frequently *heighten* irritability;[2] *i. e.*, expressed in terms of the membrane theory of stimulation, they increase the readiness with which the permeability—and hence the electrical polarization—of the plasma membrane undergoes change. It seems clear that for irritable tissues the state of the lipoids in the plasma membrane largely determines the readiness with which changes of permeability—and of the dependent electrical polarization—are induced by external agencies. Slight permeation of the lipoids with a lipoid-solvent like ether apparently often facilitates such changes and hence increases irritability; the presence of more lipoid-solvent renders a change of permeability difficult,[1] hence the protective or anæsthetic action; while concentrated solutions of lipoid-solvents disrupt the membrane and produce cytolytic or irreversible alterations in the cells; hence such substances in higher concentrations are markedly toxic.

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[1] R. S. Lillie, *American Journal of Physiology*, 1912, Vol. 29, p. 372.

[2] For various instances of this effect cf. the references cited in my recent paper in *American Journal of Physiology*, 1912, Vol. 29, p. 374. The neuromuscular system of marine animals also shows it; *e. g.*, Bethe found that alcohol (0.5 per cent. in sea-water) decidedly increased the mechanical irritability of the isolated central portion of the medusa *Cotulorrhiza;* cf. "Allgemeine Anatomie und Physiologie des Nervensystems." Leipzig, 1903, p. 359.

On the assumption that lipoid-solvents influence the plasma membranes of egg cells in essentially the same manner as those of irritable tissues, the above action of ether on abnormal egg cells becomes more readily intelligible; it falls, in fact, into the same essential category with the facts just cited. There is a close analogy between the stimulation of irritable tissues and the initiation of cleavage in egg cells; the primary or critical change in both cases appears to be a temporary and reversible increase in the permeability of the plasma membrane, with accompanying changes in the electrical polarization of the latter.[2] This analogy suggests that the irresponsive condition of the above "over-ripe" starfish eggs is essentially the symptom or expression of an abnormal condition of the plasma membrane. Apparently the latter has in these eggs become *abnormally resistant to changes of permeability;* hence the eggs are irresponsive to the spermatozoön (whose primary action is to increase permeability); hence also they show heightened resistance to cytolytic action—which also depends on increase in surface permeability; this is shown by the slowness with which they undergo the typical post-maturational cytolysis, and also by their increased resistance to pure isotonic sodium chloride solution. If this interpretation is correct, the favorable action of weak ether solution consists essentially in altering the plasma membrane and rendering it more susceptible to the action of permeability-increasing (and hence depolarizing) agencies—*i. e.*, *more irritable*, on the above-mentioned analogy with irritable tissues. Through this means the plasma membrane is restored to an approximately normal condition of responsiveness; the sperm then exhibits its normal action. It is evident that this hypothesis also implies that the other changes in the egg expressive of increased permeability should, after the ether treatment, also follow an approximately normal course. This in in fact the case as regards the post-maturational cytolysis; this change is delayed in the above abnormal eggs, as already described; but it is found to take place in a normal manner in the ether-treated unfertilized eggs. The following descriptions will illustrate both of these effects.

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[1] This is very clearly shown in the larvæ of *Arenicola;* cf. the paper just cited, p. 380 ff.

[2] I have discussed the probable basis of this resemblance at some length in an earlier paper in the BIOLOGICAL BULLETIN, 1909, Vol. 17, pp. 20 ff. The title of Loeb's recent book, "Entwicklungserregung des tierischen Eies," also emphasizes this analogy.

It should first be noted that the degree to which eggs abnormally resistant to fertilization may be rendered normal by the ether treatment is variable. In some of my last summer's experiments the difference between the ether-treated and the untreated eggs of the same lot was slight; in others the contrast was most striking. The degree of resistance to the post-maturational cytolysis is similarly variable. In general it was observed that eggs which showed the most pronounced delay in the onset of this latter change were most readily brought into a normally responsive condition—or "rejuvenated"—by ether. The following series of experiments with three separate lots of starfish eggs—all of which failed with a few exceptions to develop to a blastula stage on simple fertilization without ether treatment—illustrates this variability, as well as the correlation between delay in the post-maturational cytolysis and the possibility of rejuvenation[1] by the ether treatment.

June 30, 1911. Three separate lots of eggs (*A*, *B*, *C*) were used; each lot consisted of the mixed eggs from several starfish. After remaining about one and a half hours in normal sea-water, eggs from each lot were transferred to sea-water containing 0.3 vol. per cent. ether. These eggs were kept in small tightly corked flasks. After 1 hour and 10 minutes in this solution part of the eggs were transferred from each flask to normal sea-water in finger bowls; after washing the eggs free from ether spermatozoa were added. The remainder of the eggs in each flask were similarly transferred to sea-water and fertilized after three and a quarter hours in the ether solution. For each lot there was a *fertilized control* consisting of eggs which had lain untreated in sea-water for about 2 hours and 45 minutes before fertilization.

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[1] I use this term because of the analogies it suggests. The eggs are in fact brought by the ether treatment into a condition which is characteristic of eggs produced in the earlier portion of the reproductive cycle. The production of hyper-resistant eggs like the above occurs late in the breeding season, and the phenomenon bears certain analogies to senescence. See below, page 345.

The characteristics and behavior of the eggs from these three lots were respectively as follows:

Lot A.—The great majority of these eggs fail to maturate. A small proportion undergo apparently normal maturation.

Unfertilized Eggs.—22 hours after removal almost all of the mature eggs show the typical opaque and coagulated protoplasm; *i. e.*, post-maturational cytolysis appears normal.

Fertilized Eggs.—Condition *ca.* 20 hours after fertilization.

1. *Untreated* (*Control*) *Eggs.*—Many of the immature eggs have typical fertilization membranes; but are otherwise unchanged. The few mature eggs are mostly dead; only one abnormal blastula was found.

2. *Ether-treated Eggs.*—(*a*) Exposed 1 hour 10 minutes. Four or five blastulæ are found in some hundred eggs; little difference from control. (*b*) Exposed 3¼ hours. Little or no improvement over control; a few blastulæ as in (*a*).

Lot B.—Most of these eggs remain immature, but about 20 per cent. undergo apparently normal maturation.

Unfertilized Eggs.—22 hours after removal from the animals most of the mature eggs are opaque and coagulated, but in many the coagulation is less advanced than in normal eggs, and in some the protoplasm remains semi-translucent.

Fertilized Eggs.—Condition *ca.* 20 hours after fertilization.

1. *Untreated* (*Control*) *Eggs.*—Almost all of the mature eggs are dead. Many immature eggs have fertilization membranes. Only two abnormal blastulæ are found in several hundred eggs.

2. *Ether-treated Eggs.*—(*a*) Exposed 1 hour 10 minutes. Improvement over the control; a large proportion (about one third) of the mature eggs have formed blastulæ, many of which have begun to gastrulate. (*b*) Exposed 3¼ hours. Also shows a marked improvement over the control, but the larvæ are fewer and less active than in 2*a*.

Lot C.—In this lot of eggs the majority show normal maturation, though a few remain immature.

Unfertilized Eggs.—After 22 hours in sea-water most of the eggs are more or less coagulated, but the degree of opacity is distinctly less than in normal eggs, and a considerable proportion remain translucent—almost like freshly shed eggs.

Fertilized Eggs.—Condition *ca.* 20 hours after fertilization.

1. *Untreated* (*Control*) *Eggs.*—Nearly all are dead. Most have membranes and show evidence of having cleaved or fragmented, but many have failed to cleave or even to form membranes. A small proportion of eggs have formed larvæ some of which appear normal: the larvæ though few are more numerous than in the controls of *A* and *B*.

2. *Ether-treated Eggs.*—(*a*) Exposed 1 hour 10 minutes. Striking contrast to control. Almost all of the mature eggs have formed active larvæ, many in the early gastrula stage and swimming at the surface of the water. (*b*) Exposed 3¼ hours. Here also the majority of eggs form larvæ, but these are largely abnormal, and relatively few gastrulæ or surface swimmers are present.

The power of development after fertilization is thus greatly increased after ether-treatment in Lots *B* and *C*, but not in Lot *A*. The mature eggs of Lots *B* and *C* show marked delay in the post-maturational cytolysis; in Lot *C* this delay is greater, and the action of the ether is correspondingly more favorable, than in Lot *B*. I have already presented evidence that eggs showing this abnormal behavior are characterized by the possession of hyper-resistant plasma membranes. It should be noted that variations in the degree of resistance of this membrane occur regularly in normal eggs. Various facts indicate that the process of maturation is constantly associated with a change in the properties of the plasma membrane. This is shown by the fact that mature eggs undergo cytolysis in 0.55*m* NaCl solution more rapidly than immature eggs;[1] also by the fact that contact with spermatozoa and various forms of artificial treatment cause the separation of the surface-film of mature eggs in the form of a fertilization membrane, a change indicating a superficial cytolytic or permeability-increasing action; while immature eggs are not normally subject to this change.[2] This difference between immature and mature eggs is a constant or physiological feature in the life history of these eggs. The difference between normal eggs and the resistant eggs under consideration is in many respects similar. For some reason the maturation process fails to bring these eggs into the normally sensitive condition in which the permeability of the membrane is readily increased. Hence fertilization is imperfect, even fertilization-membranes failing to form in some cases; in others membranes are formed and cleavage begins, but the latter is characteristically irregular and fails to proceed far. According to this view the failure of development is due not to defective organization of the protoplasm, but simply to the existence of an abnormally resistant plasma membrane. The action of ether consists in restoring the membrane to its normal condition. The response to the spermatozoön then becomes normal.

The experimental evidence in favor of this hypothesis consists at present simply in the fact that such abnormal eggs are rendered normal by ether treatment not only in regard to their response to fertilization, but also in regard to the rate and character of the spontaneous post-maturational cytolysis; *i. e.*, they undergo complete coagulation within eighteen hours or less, precisely as do normal eggs. Whether the resistance to cytolysis by salt solutions is also decreased I have not yet determined; but the decrease in the resistance to the post-maturational cytolysis—a change supposedly due to the action of certain protoplasmic oxidation products upon the plasma membrane[1]—is clear evidence that the membrane has been brought into a condition more nearly approaching the normal. The experiments to be described show that a return of the normal responsiveness to the spermatozoön is closely correlated with a return of the normal behavior with respect to this spontaneous oxidative cytolysis. In other words, the plasma membranes of the ether-treated "rejuvenated" eggs undergo breakdown in the manner and at the time characteristic of normal eggs.

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[1] R. S. Lillie, *American Journal of Physiology*, 1912, Vol. 30, p. —.

[2] Cf. J. Loeb's experiments on the eggs of *Asterina*, University of California Publications, Physiology, 1905, Vol. 2, p. 150. Spermatozoa and artificial membrane-forming agencies may however produce typical membranes in abnormal immature starfish eggs. Cf. *Journal of Experimental Zoölogy*, 1908, Vol. 5, p. 407.

The following record gives the description of two typical experiments.

July 6, 1911. Eggs were removed at 11:00 A.M. from two lots of starfish, *A* and *B*. In both lots a good proportion of eggs underwent apparently normal maturation. Eggs from each lot were divided into two portions. One portion remained in sea-water; and about four hours after removal from the animals part of these eggs were fertilized; the rest remained unfertilized. The other portion was transferred, two hours after removal, to sea-water containing 0.3 vol. per cent. ether; in this solution they remained for one hour and thirty-five minutes; they were then returned to sea-water; to part of these eggs spermatozoa were added, the rest remained unfertilized. The ether-treated and the untreated eggs were fertilized at the same time. The two lots *A* and *B* were treated alike so far as possible. The results of these experiments were as follows:

LOT A. The following was the condition of the eggs *ca.* 22 hours after removal:

1. *Untreated Eggs.* (*a*) *Unfertilized.*—Most mature eggs are coagulated but to a varying degree; some are only slightly darkened, and in a fair proportion the protoplasm remains semi-translucent.

(*b*) *Fertilized.*—All of the mature eggs have formed membranes and most have undergone cleavage or irregular fragmentation; but many remain uncleaved; no blastulæ are present.

2. *Ether-treated Eggs.*—(In 0.3 vol. per cent. ether from 1.00 to 2.35 P.M.)

(*a*) *Unfertilized.*—All mature eggs are completely and uniformly coagulated; there are no partly coagulated or semi-translucent eggs.

(*b*) *Fertilized.*—Decided contrast to 1*b*. Most eggs are dead, but among these there are no uncleaved eggs; numerous blastulæ and gastrulæ are present, many swimming at the surface.

LOT B.—(The condition of the eggs *ca.* 22 hours after removal.)

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[1] Since the change is greatly retarded in oxygen-free or cyanide-containing sea-water. Cf. J. Loeb, *Archiv für die gesammte Physiologie*, 1902, *loc. cit.*

1. *Untreated eggs.*—(*a*) *Unfertilized.*—The degree of post-maturation coagulation varies here as in Lot *A*, but a larger proportion of eggs remain semi-transclucent, and many are virtually unchanged in appearance.

(*b*) *Fertilized.*—Most eggs have formed membranes and cleaved or fragmented; a number form membranes but fail to cleave. A few feeble blastulæ are present—a fraction of 1 per cent.; no surface swimmers.

2. *Ether-treated eggs.*—(In 0.3 vol. per cent. ether from 1.00 to 2.35 P.M.)

(*a*) *Unfertilized.*—Marked contraxt to 1*a*. All mature eggs are completely coagulated—opaque, compact looking, without membranes.

(*b*) *Fertilized.*—A large number of active blastulæ and gastrulæ are present, many swimming at the surface. No uncleaved eggs are present, though a good many have died in early stages. More favorable than *A*, 2 (*b*).

The return of the normal responsiveness to fertilization and normal power of development is thus associated with a return of the normal rate of post-maturational cytolysis. This observation was made in six out of nine experiments, at different times and with different lots of eggs, in which ether-treatment led to marked increase in the proportion of eggs undergoing favorable development. In three of the earlier experiments the behavior of the ether-treated unfertilized eggs was not observed; but in all of the six cases where both observations were made concurrently this correlation held. As already mentioned, the degree of improvement effected by the ether has been variable, in correspondence with the degree of abnormality in the eggs. In all of the cases in which the improvement was decided, as in some of those described above, the untreated mature eggs proved largely refractory toward both cytolysis and fertilization; while after the treatment with ether the eggs showed in both respects a behavior approaching the normal. In three other lots of abnormal eggs treatment with ether had no appreciable effect either in accelerating cytolysis or in increasing the proportion of favorably developing eggs; while in one case in which a considerable proportion of untreated eggs developed favorably—about one third forming larvæ—the ether-treated eggs were somewhat *less* favorable than the untreated; in this series the unfertilized eggs, both treated and untreated, showed an apparently normal spontaneous cytolysis. Probably the plasma membranes of these eggs were over-susceptible rather than under-susceptible to increase of permeability. The possible existence of both kinds of abnormalities must be recognized.

Deviation in either direction from the physiological norm would presumably impair the power of development.

General Discussion.

I shall now discuss somewhat more fully the general physiological significance of the above abnormalities and their relations to analogous conditions elsewhere. The above condition, described in general terms, is essentially one of lowered susceptibility to agencies which ordinarily call forth a definite response. Similar conditions exist in other cells and tissues. There are also cases where a tissue is normally irresponsive to certain agencies or conditions, to which however it may be rendered responsive by certain forms of artificial treatment.[1] It seems probable that in all of these cases the condition of the plasma membrane is the essential factor which determines whether the cell or tissue responds to the agency in question or not. This structure, as the most external layer of the cell, is the part most accessible to artificial modification; and if its condition of permeability and electrical polarization plays the controlling rôle in cell-processes which modern investigation tends more and more to indicate, knowledge of the means by which its properties may be altered at will becomes a matter of the highest importance for both the theoretical and the practical aspects of biology.

The abnormalities under consideration appear typically in the eggs of *Asterias forbesii* toward the close of the breeding season. Eggs are abundant at Woods Hole in early June.[2] During the greater part of this month they exhibit as a rule a normal response to fertilization; and if left unfertilized in sea-water at 20° they undergo the above described coagulative cytolysis within 12 to 15 hours or less. Toward the end of June eggs become fewer and more variable in quality, many fail to maturate and the mature eggs on fertilization tend to cleave irregularly and largely die before reaching the blastula stage; a large proportion of eggs show the abnormalities described above; increased resistance to fertilization and to cytolytic alteration is especially characteristic and indicates that the plasma membrane has become abnormally resistant to changes in permeability.

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[1] Instances of this are seen in various phenomena of sensitization. A good instance is the hypersensitiveness to contact induced in frog's skeletal muscle by isotonic solutions of sodium citrate, tartrate, sulphate, and certain other salts. Cf. J. Loeb, *American Journal of Physiology*, 1901, Vol. 5, p. 362.

[2] In former years a considerable proportion of starfish collected in August and September have yielded numerous normal eggs. Probably these starfish were of a different species from the above—presumably *A. vulgaris*. During the last few years this form seems to have become rare in the neighborhood of Woods Hole, and eggs have been difficult to obtain later than June. Two species of *Asterias*, *forbesii* and *vulgaris*, are recognized as occurring in this region; cf. H. L. Clark, Bulletin of the U. S. Fish Commission, 1902, p. 552.

I have described the peculiarities of these eggs in sufficient detail above, and have already briefly discussed the physiological nature of the abnormalities. The failure to respond normally to fertilization becomes intelligible on the hypothesis that the essential or critical event in the initiation of cell-division is a temporary and reversible increase in the ionic permeability of the plasma membrane. Such a change involves a decrease in the electrical polarization of the membrane, and it appears probable—as in the analogous case of muscle and nerve—that this change of polarization, and not the mere increase of permeability as such, is the critical event which initiates the rhythmical series of physical and chemical processes of which cleavage is the normal expression.[1] In order that the egg may respond to the contact and entrance of the spermatozoön in a normal manner, its plasma membrane must have a certain definite physico-chemical constitution such that the substances transmitted by the spermatozoön[2] may effect an increase of permeability which in rate and degree approximates a certain norm. This implies a certain degree of resistance to change of permeability; if this resistance is abnormally great, the response fails to occur or is imperfect; if abnormally low, the spermatozoön effects too great and too lasting an increase in permeability resulting in early death or cytolysis—just as occurs in eggs subjected to a simple membrane-forming treatment without subsequent exposure to hypertonic sea-water.[1] Eggs which begin cleavage, but fragment and break down before proceeding far in development, possibly belong to this latter class. If normal development is to follow fertilization, the properties of the plasma membrane cannot, on the present hypothesis, depart widely from a constant mean or physiological norm.

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[1] The astral radiations of mitosis are probably the expression of potential differences between different regions of the cell, arising in consequence of altered polarization of the limiting membranes. The alternate appearance and disappearance of the radiations indicates a rhythm of changing polarization of the limiting membranes. For a further discussion cf. my papers in BIOLOGICAL BULLETIN, 1909, *loc. cit.*; *Amer. Jour. Physiol.*, 1910, Vol. 26, pp. 128 ff.; *Journal of Morphology*, 1911, Vol. 22, p. 711.

[2] According to Loeb, certain lysin-like bodies (cf. "Entwicklungserregung," p. 247). I have suggested calling these "membranolysins" to indicate the essential nature of their action.

The failure of the above eggs to respond normally to fertilization, as also their resistance to cytolysis, is thus to be regarded as the expression of a highly resistant condition of the plasma membrane. The latter fails readily to undergo the increase of permeability essential to these changes,—probably because of abnormalities in the nature, state, or proportions of its chemical constituents. The essential effect of the treatment with ether is to restore the normal properties of the membrane. There is, however, no reason to believe that this effect is specific to ether. In some of my last summer's experiments a similar though less favorable effect was produced by exposure to isotonic sodium chloride solution and—in one case—to a 0.1 per cent. solution of chloral hydrate in sea-water. In its general form the problem relates to the essential nature of the modification which these substances induce in the egg, and by which the latter is brought from an irresponsive condition into one in which it shows a normal response. Light is thrown on this problem by the conditions in irritable tissues such as muscle and nerve.

A close analogy exists between the initiation of cell-division in eggs or other resting cells, and the response of an irritable tissue to stimulation. In both cases the initial or critical event is apparently a temporary increase in surface-permeability, with accompanying changes in the electrical polarization of the limiting membranes. The means by which refractory eggs may be rendered normally responsive is thus analogous to that by which the responsiveness of muscle and nerve to stimulation may be increased. An irresponsive condition in living muscle and nerve may be due to anæsthesia, fatigue, electrotonus, toxic action, or other changes of state. The stimulating action of small doses of alcohol[1] and other narcotics during fatigue suggests an analogy which is probably not without significance. It is known that traces of various lipoid-solvent substances very generally increase irritability, or the rate of spontaneous activity, in the most various cells and tissues (leucocytes, cilia, the heart, etc.).[2] The increased responsiveness of the above eggs after ether treatment is a phenomenon of the same general kind. It would appear that the condition of the lipoids in cells determines the readiness of response or the rate of spontaneous activity; and that slight impregnation of the lipoids in the membrane with a lipoid-solvent facilitates in this structure the alteration which conditions the permeability-increase and polarization-change of stimulation.

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[1] The second part of the treatment appears to effect a return of the permeability—which has been increased by the membrane-forming treatment—to the normal (cf. *Amer. Jour. Physiol.*, 1911, Vol. 27, p. 289). Godlewski (*Archiv für Entwicklungsmechanik*, 1911, Vol. 33, p. 225) has independently reached a similar conclusion with regard to the essential nature of the effect produced by the hypertonic sea-water.

Certain salts markedly increase the irritability of muscle and nerve,—*i. e.*, induce sensitization.[3] Treatment with salt solutions may also restore fatigued muscles to an irritable condition. Frog's skeletal muscles immersed in isotonic sodium chloride solution and made to contract by successive electrical stimuli until irresponsive promptly recover irritability if immersed in isotonic sodium bromide, nitrate or iodide solutions. Sodium iodide restores irritability to muscles which have been fatigued in sodium bromide or nitrate solutions, but chloride has no such action; *i. e.*, the order of the salts cannot be reversed. The restorative effect is rapid, and evidently depends on a surface action, the colloids of the membrane being apparently brought into a condition favorable for stimulation—apparently a condition of increased dispersion.[4] As already described, sodium chloride solution may produce an analogous increase of responsiveness in refractory starfish eggs. Schwarz's observations, as well as my own with abnormal starfish eggs, thus belong to that general class of cases in which the responsiveness of cells is increased by treatment with salts or low concentrations of lipoid-solvents. The response of voluntary muscle to various forms of chemical stimulation may be increased by brief immersion in isotonic solutions of various sodium salts; in the case of salts which do not precipitate calcium, this sensitizing action increases with variation in the nature of the anion in the following general order: $Cl < Br < NO_3 < ClO_3 < CNS$ and I, an order corresponding to the order of increasing effectiveness in promoting colloidal dispersion.[1] Interpreted in terms of the membrane theory, these facts mean that the readiness with which the plasma membrane undergoes increase in permeability may be increased either by altering the general state of the colloids in the membrane, or by slightly altering that of the lipoids alone.

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[1] The action of small quantities of alcohol in counteracting fatigue in excised frog muscles has been well shown by Lee and Salant, *Amer. Jour. Physiol.*, 1902, Vol. 8, p. 61.

[2] Cf. footnote 2 , page 331 .

[3] Cf. footnote 1 , page 338 .

[4] C. Schwarz, *Archiv für die gesammte Physiologie*, 1907, Vol. 117, p. 161.

We conclude that the effect produced by salts and weak ether solutions in increasing the responsiveness of refractory eggs to fertilization is comparable with the sensitization of irritable tissues by these substances; also that in both cases the essential change consists in an increase in the readiness with which the plasma membrane undergoes the critical change of permeability and of electrical polarization.

It is to be noted that the resistance of eggs to fertilization by foreign sperm may also be decreased by chemical treatment, as Loeb discovered several years ago.[2] Heightening the alkalinity of the medium has this effect. This characteristic and striking effect is probably an expression of a very general action of weak alkali. Many facts indicate that slight increase in the alkalinity of the medium usually increases the readiness with which the permeability of cells is altered: cell-division is accelerated, the irritability of irritable tissues and the rate of activity of automatic tissues is increased, the cytolytic action of salt solutions is accelerated, and in unfertilized eggs membrane formation and the initiation of cleavage may be induced.[3] It remains to be determined whether alkali as well as ether can overcome the resistance of over-ripe starfish eggs to fertilization by their own sperm; also whether treatment with weak solutions of ether or other lipoid-modifying substances, as well as with weak alkali, can render cross-fertilization possible. The case of the eggs of hermaphrodite animals, which are irresponsive to sperm from the same individual but not to that of other individuals, may possibly belong in part to the present category. Morgan[1] found that the eggs of *Ciona*, which furnish a typical instance of this behavior, could be fertilized by spermatozoa from the same individual in weak solutions of ether, ammonia or alcohol; but he is inclined to attribute the effect to the stimulating action of these substances on the sperm, rather than to an alteration of the egg. In one experiment, however (p. 147), in which the spermatozoa alone were treated with ether before adding to the egg, few eggs were fertilized, while when both eggs and sperm were so treated the percentage of fertilization was high (in one case 85 per cent. as compared with 4 per cent.). This experiment suggests that the ether produces its effect not merely by increasing the motility of the sperm, but also by altering the condition of the egg, as in the case of *Asterias*. Another interesting case in which the egg is rendered refractory to fertilization has recently been described by Godlewski.[2] If the spermatozoa of *Chætopterus* and of *Sphærechinus* are mixed, both are found after a few minutes to have completely lost the power of fertilizing the eggs of *Sphærechinus*.[3] Eggs left exposed to this sperm mixture are at first unaffected, but in the course of half an hour they become so modified that fertilization with normal *Sphærechinus* sperm is impossible. The egg is thus deprived of the power of response to its own spermatozoa.[1] Whether this condition of irresponsiveness (which is comparable to paralysis) may be removed by artificial treatment such as the above has apparently not been determined; but from the analogies with the conditions just described there is every reason to believe that this would readily be found possible.[2]

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[1] R. S. Lillie, *Proceedings of the Society for Experimental Biology and Medicine*, New York, 1910, Vol. 7, p. 170.

[2] J. Loeb, University of California Publications, Physiology, 1903, Vol. 1, p. 1.

[3] Cf. J. Loeb, *Archiv für die gesammte Physiologie*, 1907, Vol. 118, p. 7.

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[1] Morgan, *Journal of Experimental Zoology*, 1904, Vol. 1, p. 135.

[2] Godlewski, *Archiv fur Entwicklungsmechanik*, 1911, Vol. 33, p. 233.

[3] The spermatozoa in this mixture retain their motility in spite of their having lost the power of affecting the egg (*loc. cit.*, p. 240). It would be interesting to know if the motility undergoes *any* decrease. Apparently the surface layer of the spermatozoon is modified, since there is no transmission of membranolysins to the egg. This would imply heightened resistance to permeability-change in the plasma membrane of the spermatozoon; and such a change would presumably involve decreased motile activity—*i. e.*, a slower rhythm in the variation of ionic permeability and polarization conditioning the movements. Various facts indicate that in normal fertilization the spermatozoön and the egg affect each other *mutually* in a somewhat similar manner, and that a cytolytic or permeability-increasing action is exercised by the egg upon the sperm as well as by sperm upon the egg. Thus the sperm usually ceases its movements soon after contact with the egg, and often only a portion enters the latter; a cytolytic or disintegrative change in the spermatozoön is thus indicated. The plasma membrane of the sperm might—as apparently in Godlewski's experiments—be rendered sufficiently hyper-resistant to prevent this mutual cytolytic action without entire loss of motility.

In conclusion I wish briefly to indicate the bearing of the above observations on the general theory of pathological alterations in cells. The conclusion that many pathological conditions have their primary origin in abnormalities of the limiting membranes of cells is an obvious corollary of any view that regards such membranes—which are essentially insulating surface-films of varying ionic permeability and electrical polarization—as largely controlling the rate and character of the cell-processes. If stimulation depends primarily on altered polarization of the plasma membrane due to increased ionic permeability, it is clear that a normal response, in the case of any cell, implies a definite condition of the membrane. If this condition is permanently altered the cell processes inevitably undergo derangement, and pathological changes follow. Such a deranged condition, if not too far advanced, may be rectified by restoring the membrane to its normal condition. How this may be accomplished is illustrated by the case of the abnormal starfish eggs described above. It is clear from the cytolytic effects produced by many toxines that they cause abnormal increase in the permeability of the membranes; and in all probability their destructive action is in many cases directly due to this surface action. The alteration caused by a toxic agent may consist primarily either in increasing or in decreasing the permeability normal to the membrane, or in altering in either direction the readiness with which the latter undergoes change. Evidently the plasma membrane, as an insulating—and hence semi-permeable—layer on the integrity of which the normal composition of the living substance depends, cannot undergo marked and prolonged increase of permeability without alteration in the nature and proportion of the cell-constituents; this involves altered chemical organization and eventual derangement of the cell-processes.[1] In most of the abnormal conditions considered above the membrane appears to have undergone the opposite kind of modification, becoming abnormally impermeable and resistant to changes of permeability. Such a condition is essentially one of irresponsiveness, and is in a sense pathological, although it differs from a condition of permanently increased permeability in involving no loss of material from the cell; hence the possibility of restoring the normal properties of the cell—by bringing the permeability again to the normal—should theoretically be greater in this class of cases than in the other.[2]

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[1] A similar and reversible effect may be produced by treating the egg with certain salt solutions, as Mathews and Newman showed some years ago for *Fundulus* eggs; cf. BIOLOGICAL BULLETIN, 1905, Vol. 9, p. 378.

[2] Evidence that this change is in fact reversible is seen in the effects of washing the eggs thoroughly in sea-water after the treatment with the sperm mixture. A partial return of responsiveness to the spermatozoön was observed (p. 236).

Perhaps the condition of the above resistant starfish eggs is not properly to be called pathological, since the change in the properties of the eggs toward the close of the breeding season is presumably a constant one, and hence normal in a physiological sense. The eggs merely become hyperresistant to fertilization; *i. e.*, with increasing age the metabolism of the ovaries undergoes alteration so as to lead to the production of eggs having more resistant membranes than before. The cycle of egg-production shortly afterwards comes to a close. The phenomenon bears in certain respects a marked resemblance to senescence, and its conditions may throw light on the physiology of the latter process. In old age the irritable tissues become less and less responsive, and the rate of metabolism is correspondingly lowered. Irresponsiveness, as already pointed out, implies a state of the plasma membrane in which changes of permeability and of electrical polarization are produced with difficulty. The increasingly resistant character of the membranes implies altered composition. Possibly the condition in over-ripe eggs, as well as in senescence, results from a progressive accumulation or adsorption at the phase-boundaries, *i. e.*, in the membranes, of materials which for some reason are not readily eliminated by the organism.[1] A similar view has recently been expressed by Child.[2] According to his theory "senescence in nature consists physiologically in a decrease in the rate of metabolism, and this is determined morphologically by the accumulation in the cell of structural obstacles to metabolism, *e. g.*, decrease in permeability, increase in density, accumulation of relatively inactive substances, etc." Minot's[3] theory that senescence is the expression of a progressively increasing differentiation of cells, *i. e.*, of an increase in the proportion of separated solid structural material, bears a certain resemblance to this view. The view which I have suggested above is distinctive only in so far as it attributes the essential change to a modification of the *membranes*. It is, theoretically at least, within the possibilities of physiological science to prevent or retard this accumulation of inert materials in the membranes and so to delay senescence. Or the already modified membranes might, if not too profoundly altered, be restored to a normal condition by certain forms of treatment. The simpler the metabolism and the less widely differentiated the tissues, the greater would appear to be the possibility of such "rejuvenescence" of the organism as a whole. In one large group, the Protozoa senescence seems not to be an inevitable occurrence; the earlier interpretation of conjugation as a process whose essential rôle is to counteract an innate tendency to senescence has been discredited by the work of Calkins and Woodruff. The conditions in Metazoa differ from those in Protozoa chiefly in their greater complexity, but probably in no other essential respect.

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[1] I have dwelt on these considerations at somewhat greater length in my earlier paper in this journal, 1909, Vol. 17, p. 197 et seq. The fundamental importance of the part which alterations of the membranes play in pathological processes was first recognized by Zangger. The following quotation will illustrate: "Die normale typische Permeabilität der Membranen ist also Voraussetzung der normalen Lebensfunktionen. Dauernd veränderte Permeabilität der Membranen bedeutet Pathologie, pathologischen Stoffwechsel," etc.: Vierteljahrsschrift d. Naturf. Ges. Zürich, 1906, Vol. 51, p. 432. Cf. also *ibid.*, 1907, 1908, and the other papers of Zangger and his students, especially Frei and Stoffel, for a fuller development of these ideas, together with experimental data bearing on the relations of membrane changes to pathological processes, immunity, and related phenomena.

[2] For further discussion of this subject cf. my earlier paper in *American Jour. Physiol.*, 1910, Vol. 26, pp. 112 et seq.

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[1] Probably certain colloidal (*i. e.*, indiffusible) and chemically refractory by-products of metabolism.

[2] Cf. Child, *Archiv für Entwicklungsmechanik*, 1911, Vol. 31, p. 537.

[3] C. S. Minot, "The Problem of Age, Growth and Death." New York and London, 1908.

THE MARINE BIOLOGICAL LABORATORY

FOURTEENTH REPORT

FOR THE YEAR 1911

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I. TRUSTEES

EX OFFICIO

F. R. LILLIE, *Director*, The University of Chicago.

GILMAN A. DREW, *Assistant Director*, University of Maine.

D. BLAKELY HOAR, *Treasurer*, 161 Devonshire Street, Boston, Mass.

THOS. H. MONTGOMERY, JR.,[1] *Clerk of the Corporation*, University of Pennsylvania.

TO SERVE UNTIL 1915

H. C. BUMPUS	University of Wisconsin.
R. A. HARPER	Columbia University.
W. A. LOCY	Northwestern University.
JACQUES LOEB	Rockefeller Institute for Medical Research.
F. P. MALL	Johns Hopkins University.
George T. MOORE	Missouri Botanical Garden, *Secretary of the Board*.
L. L. NUNN	Telluride, Colo.
JOHN C. PHILLIPS	299 Berkeley Street, Boston, Mass.

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[1] Deceased.

TO SERVE UNTIL 1914

Cornelia M. Clapp	Mount Holyoke College.
E. G. Conklin	Princeton University.
Ross G. Harrison	Yale University.
Camillus G. Kidder	27 William Street, New York City.
M. M. Metcalf	Oberlin College.
William Patten	Dartmouth College.
Jacob Reighard	University of Michigan.
W. B. Scott	Princeton University.

TO SERVE UNTIL 1913

S. F. Clarke	Williams College.
Charles Coolidge	Ames Building, Boston, Mass.
C. R. Crane	2559 Michigan Boulevard, Chicago, Ill., *President of the Board.*
Alfred G. Mayer	Carnegie Institution.
T. H. Morgan	Columbia University.
Erwin F. Smith	United States Department of Agriculture.
E. B. Wilson	Columbia University.

TO SERVE UNTIL 1912

M. J. Greenman	Wistar Institute of Anatomy and Biology.
C. W. Hargitt	Syracuse University.
H. S. Jennings	Johns Hopkins University.
George Lefevre	University of Missouri.
A. P. Mathews	The University of Chicago.
G. H. Parker	Harvard University.

II. ACT OF INCORPORATION

No. 3170.

COMMONWEALTH OF MASSACHUSETTS

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns, Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

Now, therefore, I, HENRY B. PIERCE, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our LORD ONE THOUSAND, EIGHT HUNDRED AND EIGHTY-EIGHT.

HENRY B. PIERCE,
Secretary of the Commonwealth.

[SEAL.]

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

I. The annual meeting of the members shall be held on the second Tuesday in August, at the Laboratory, in Woods Hole, Mass., at 12 o'clock noon, in each year, and at such meeting the members shall choose by ballot a Treasurer and a Clerk, who shall be, *ex officio*, members of the Board of Trustees, and Trustees as hereinafter provided. At the annual meeting to be held in 1897, not more than twenty-four Trustees shall be chosen, who shall be divided into four classes, to serve one, two, three, and four years, respectively, and thereafter not more than eight Trustees shall be chosen annually for the term of four years. These officers shall hold their respective offices until others are chosen and qualified in their stead. The Director and Assistant Director, who shall be chosen by the Trustees, shall also be Trustees, *ex officio*.

II. Special meetings of the members may be called by the Trustees, to be held in Boston or in Woods Hole at such time and place as may be designated.

III. The Clerk shall give notice of meetings of the members by publication in some daily newspaper published in Boston at least fifteen days before such meeting, and in case of a special meeting the notice shall state the purpose for which it is called.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. The Trustees shall have the control and management of the affairs of the Corporation; they shall present a report of its condition at every annual meeting; they shall elect one of their number President and may choose such other officers and agents as they may think best; they may fix the compensation and define the duties of all the officers and agents; and may remove them, or any of them, except those chosen by the members, at any time; they may fill vacancies occurring in any manner in their own number or in any of the offices. They shall from time to time elect members to the Corporation upon such terms and conditions as they may think best.

VI. Meetings of the Trustees shall be called by the President, or by any two Trustees, and the Secretary shall give notice thereof by written or printed notice sent to each Trustee by mail, postpaid. Seven Trustees shall constitute a quorum for the transaction of business. The Board of Trustees shall have power to choose an Executive Committee from their own number, and to delegate to such Committee such of their own powers as they may deem expedient.

VII. The President shall annually appoint two Trustees, who shall constitute a committee on finance, to examine from time to time the books and accounts of the Treasurer, and to audit his accounts at the close of the year. No investments of the funds of the Corporation shall be made by the Treasurer except approved by the finance committee in writing.

VIII. The consent of every Trustee shall be necessary to a dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be given to the Boston Society of Natural History, or some similar public institution, on such terms as may then be agreed upon.

IX. These By-Laws may be altered at any meeting of the Trustees, provided that the notice of such meeting shall state that an alteration of the By-Laws will be acted upon.

X. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

IV. TREASURER'S REPORT

For the Year Ending December 31, 1911

Income

Annual dues		$ 728.00	
Donations		13,412.00	
Homestead, general account (net)		1,111.93	
Miscellaneous:			
Interest on deposits	$97.06		
Rent of microscopes	9.90		
Use of drain (4 years)	16.00	122.96	
Supply department		10,303.61	
Tuitions		4,574.99	$30,253.49

Expenses[1]

Administration	$3,044.22
Advertising and printing	137.26
Bath house	96.91
Biological Bulletin (net)	1,082.66
Boats	6,003.45
Chemical department	838.44
Dormitories	72.97
Fish-trap	239.66
Homestead icehouse	609.92
Homestead shop	262.91
Instructors' salaries	3,175.00
Interest	150.00
Lectures	25.96
Library	1,297.71
Maintenance of buildings and grounds	1,879.00
Mosquito fund	280.47
Real estate	5,100.00
Scientific instruments	504.19

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[1] Owing to a change in classification of expenses, the amounts charged the various accounts are not comparable with the reports of previous years.

Sundries	219.81	
Supply department	8,008.53	$33,029.07[1]

Itemized List of Sundry Expenses for the Year 1911

Expenses of W. C. Curtis	$29.79	
Howes' bills	1.84	
Teaming and freight	2.57	
Express	19.00	
Exchange on checks	21.36	
Care of lot in cemetery	2.00	
Gasoline for gas machine	31.90	
Palmer & Laughlin (order W. C. Curtis)	6.00	
S. I. Snow (sundry bills)	3.67	
Blades	.50	
U. S. Powers (sundry bills)	2.10	
Services John G. Hubbard	60.00	
Ice	11.25	
John J. Veeder	.50	
Trustees' dinner	17.00	
E. E. Swift & Son	2.57	
Carpenter	13.74	
Charles J. Grinnell	1.35	
John F. Phillips (teaming)	5.30	
Premium on bond on alcohol	7.50	
Recording deed	.89	
Recording declaration of trust	.79	
Express on pay-rolls to Woods Hole	5.25	
Pay-roll envelopes	.29	
Telegram	.30	
Tracing paper	3.60	
Carpenter (botany department)	.45	$251.51

Credit

Petty cash at Woods Hole for 1910	$ 1.00	
Reprints	5.30	
Harvard Apparatus Company (check sent by error last year)	25.40	31.70
		$219.81

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[1] On January 1, 1911, the Laboratory had cash on hand $2,719.17; the overdraft for the year was therefore $56.41, which has since been adjusted.

MOSQUITO FUND

Receipts

1910	Miss S. B. Fay	$100.00	
	Joseph Fay, Jr.	100.00	
	A. C. Harrison	100.00	
	H. K. Dyer	100.00	
1911	Charles R. Crane	100.00	$500.00

Payments

1910	Services H. H. Brehme	$ 50.00	
	3 pair rubber boots	19.50	
	Labor (regular pay-roll)	30.00	
	Nets	1.47	
1911	Oil	5.40	
	Labor digging ditch	197.40	
	Carting	73.20	
	Spray for hose	9.72	
	Dr. Drew's expenses	11.13	
	T. E. Howes, June account	6.50	
	E. C. Brown Co.	.42	
	John F. Phillips	1.70	
	Labor (regular pay-roll)	75.00	481.44
			$18.56

MARINE BIOLOGICAL LABORATORY INVESTMENTS

JANUARY 1, 1912

RESERVE FUND

Amount of fund December 1, 1899	$4,553.14	
Received from life memberships	600.00	
Income to January 1, 1912	2,292.15	
Gain from sale of securities and rights	372.38	
	7,817.67	
Paid for current expenses of Laboratory	6,000.00	$1,817.67
Reserve Fund now consists of the following:		
$3,000 Am. Tel. & Tel. Co. 4s cost	$2,921.25	
5 shs. Am. Smelting & Refining Co. Pfd. cost	732.00	
5 shs. General Electric Co.	756.25	
14 shs. United Shoe Machinery Corp. Pfd. cost	393.75	
Cash	14.42	
	4,817.67	
Part of the above stocks and bonds are held as collateral for loan of	3,000.00	$1,817.67

LIBRARY FUND

Amount of fund December 1, 1899	$ 866.15	
Income to January 1, 1912	780.47	
Gain from sale of securities and rights	96.39	$1,743.01
Library Fund now consists of the following:		
3 shs. Am. Tel. & Tel. Co. cost	$ 383.25	
4/5 of $1,000 Am. Tel. & Tel. Co. 4s cost	779.00	
1 sh. Am. Smelting & Refining Co. Pfd. cost	122.00	
2 shs. General Electric Co. cost	302.50	
5 shs. United Shoe Mach. Corp. Pfd. cost	140.63	
Cash	15.63	$1,743.01

Lucretia Crocker Fund

Amount of fund December 1, 1899	$2,500.00	
Income after paying students' fees	557.91	
Sale of rights	8.79	$3,066.70

Lucretia Crocker Fund now consists of the following:

18 shs. Vermont & Mass. R. R. Co. cost	$2,416.50	
1 sh. West End Street R'y Co. cost	83.00	
1 sh. Am. Tel. & Tel. Co. cost	127.75	
1/5 of $1,000 Am. Tel. & Tel. Co. 4s cost	194.75	
1 sh. General Electric Co.	151.25	
Cash	93.45	$3,066.70

V. THE DIRECTOR'S REPORT

To the Trustees of the Marine Biological Laboratory:

Gentlemen: The session just closed is the twenty-fourth continuous session of the Laboratory. The attendance of investigators was larger than ever before in the history of the Institution, and the total attendance the greatest since 1902 when the policy of restricting instruction was first definitely established. The principle of coöperation, placed at the foundation of the Laboratory by Professor Whitman, has never more fully justified itself; and it is a pleasure to testify to the generous spirit in which the corporation, the board of trustees, the staff and the employees of the institution have labored for its welfare. Through Mr. Crane's generosity the Laboratory has been presented with the Kidder Annex property, a lot of land situated between the botanical laboratory and the main building; and with more than three fourths of the shares of the Woods Hole Yacht Club property adjoining and continuing our frontage on the harbor. These additions practically complete the arrangements necessary for the next forward step.

We note with regret the resignation of Professor Nathaniel L. Britton from the board of trustees, presented at the summer meeting, owing to his inability to take an active share in affairs, and we extend cordial greetings to Professor R. A. Harper, of Columbia University, a newly elected member of the board, whose coöperation will greatly strengthen our work, especially on the botanical side. The board of trustees remains otherwise unchanged. There are three vacancies in the membership which it is the province of the board to fill. Steps should be taken to secure the best men to fill these vacancies.

Professor Curtis resigned as head of instruction in invertebrate zoölogy at the close of the summer, after four years of service, during which he successfully maintained the best traditions of the course. Particular importance attaches to this course as the first to be established in the Laboratory and as lying at the foundation of the more advanced work. The directors have therefore considered the question of a successor to Professor Curtis with great care, and they are happy to be able to announce that Professor Caswell Grave, of Johns Hopkins University, has accepted the appointment as Professor Curtis' successor. We believe that no better selection could have been made. Professor Grave is given a free hand in the selection of other instructors in the course.

The attendance in 1911 taxed the resources of the Laboratory to the utmost. The number of investigators was 82 during the entire season and of students 65, a total of 147. For comparison I give the figures since 1903:

	1903	1904	1905	1906	1907	1908	1909	1910	1911
Investigators..	76	51	68	68	60	52	66	62	82
Students......	54	51	57	41	47	48	63	64	65
Total	130	102	125	109	107	100	129	126	147

But the actual increase in the number of investigators does not tell the full story. For several years there has been a steadily growing tendency on the part of workers at the Laboratory to make Woods Hole their regular summer home and to purchase houses there. The body of workers at the Laboratory has thus become not only larger, but more constant in attendance; the increase of attendance this year is therefore to be regarded as normal, not due to exceptional causes. That the Laboratory should come to be regarded as their regular summer home and working place by so large a number of prominent naturalists must be a source of gratification to all the members of the board; while it serves at the same time to emphasize anew the need of greatly increased accommodations. Such over-crowding as existed for a considerable part of last summer is certainly undesirable and its continuance for any considerable length of time could not be anticipated with composure. Neither do we wish to restrict the policy of hospitality which has been so characteristic of the Laboratory in the past.

The proposed new building is, therefore, a necessity for working space alone. And it is equally important for two other reasons:

first, to provide for the development of a more adequate library, which will remove one of the most serious limitations of our research facilities, and second, to provide more commodious quarters for certain types of research, especially in experimental lines. For the season of 1912 some additional working space will be available in the Kidder Annex, so that it may be hoped that the crowding will be no worse next year than it was this.

During the last five years there has been a steady increase in the number of subscribing institutions, from 16 in 1907 to 25 in 1911, and on the whole there is reason to believe that the present list contains fewer subscriptions for the year only than ever before; there are, moreover, indications that the increase in number may be expected to continue for some years to come. A new form of arrangement was entered into this year with the Rockefeller Institute for Medical Research of New York City, under the terms of which the Rockefeller Institute has erected a small laboratory for the use of Professor Jacques Loeb on land of the Marine Biological Laboratory, but receives other facilities and service on precisely the same terms as other co-operating institutions. Without the additional laboratory space thus provided, it would have been necessary to refuse accommodations to a number of investigators. The director has also been notified of the endowment of a scholarship in Normal College, New York City, in memory of Else Seringhaus, formerly a student at the Marine Biological Laboratory for several years. The income of the fund of $1,000.00, to be known as the Else Seringhaus Scholarship, is to be applied to the payment of tuition fees for one student each year at the Marine Biological Laboratory or elsewhere, as may be designated by the committee of award.

Following the recommendation in the director's report for 1910, Professor Gilman A. Drew was appointed resident assistant director at the summer meeting of the Board of Trustees. This step was taken none too soon, as the burden of administration of Laboratory affairs, which has been largely carried by Professor Drew for several years, in addition to regular university work, was already much too great; the Laboratory needs such service as Professor Drew's experience and character fit him so eminently well to render, and we can now face the growing complexities of the Laboratory with confidence and more ease.

Among additions to the equipment of the Laboratory during the year may be noted a large motor boat, a Zeiss microscope of the best and newest model and a new microtome. The mess did an unusually large business and exhibits an unexpectedly large surplus, all of which is to be turned back into improvements in its equipment and service. Among these may be mentioned a new ice-house costing $600.00 and a work-shop costing $250.00 already completed. The supply department has made its usual gain in business transacted, and it is worth noting that under Mr. Gray's management this business has increased from $5,616.54 in 1906 to $10,303.61 in 1911.

Our thanks are again due Mr. Crane for the donations which have enabled us to maintain the work of the Laboratory on an efficient basis; and for the presentation of additional stock of the Yacht Club, and the Kidder Annex property.

There are appended as parts of this report the names of the staff for 1911, a list of investigators and students with a tabular view of attendance since 1908, and lists of subscribing institutions, of the evening lectures for 1911, and of the members of the corporation.

1. THE STAFF.

F. R. LILLIE, Director,

Professor of Embryology and Chairman of the Department of Zoölogy, The University of Chicago.

GILMAN A. DREW, Assistant Director,

Professor of Biology, University of Maine.

ZOÖLOGY

I. INVESTIGATION

Zoölogy and Embryology

Gary N. Calkins	Professor of Protozoölogy, Columbia University.
E. G. Conklin	Professor of Zoölogy, Princeton University.
Gilman A. Drew	Professor of Biology, University of Maine.
George Lefevre	Professor of Zoölogy, University of Missouri.
Frank R. Lillie	Professor of Embryology, The University of Chicago.
T. H. Montgomery, Jr.	Professor of Zoölogy, University of Pennsylvania.
T. H. Morgan	Professor of Experimental Zoölogy, Columbia University.
E. B. Wilson	Professor of Zoölogy, Columbia University.

II. INSTRUCTION

Winterton C. Curtis	Professor of Zoölogy, University of Missouri.
Paul M. Rea	Professor of Biology, College of Charleston, and Director of the Charleston Museum.
Edward E. Wildman	Central High School, Philadelphia.
John W. Scott	Westport High School, Kansas City.
G. S. Dodds	Professor of Biology, St. Louis University.
J. F. Abbott	Professor of Zoölogy, Washington University.

EMBRYOLOGY

I. INVESTIGATION. (See Zoölogy)

II. INSTRUCTION

Gilman A. Drew......Professor of Biology, University of Maine.

Lorande L. Woodruff..Assistant Professor of Biology, Yale University.

William E. Kellicott..Professor of Biology, Goucher College.

Robert A. Budington...Associate Professor of Zoölogy, Oberlin College.

PHYSIOLOGY

I. INVESTIGATION

Albert P. Mathews....Professor of Physiological Chemistry, The University of Chicago.

R. S. Lillie............Instructor in Comparative Physiology, University of Pennsylvania.

Harold C. Bradley.....Assistant Professor of Physiological Chemistry, University of Wisconsin.

II. INSTRUCTION

H. H. Newman.........Professor of Zoölogy, University of Texas.

Charles G. Rogers.....Associate Professor of Physiology, Syracuse University.

F. H. Pike.............Instructor in Physiology, The University of Chicago.

PHILOSOPHICAL ASPECTS OF BIOLOGY AND ALLIED SCIENCES

LECTURES

Edward G. Spaulding...Assistant Professor of Philosophy, Princeton University.

BOTANY

George T. Moore......Professor of Plant Physiology and Applied Botany, Washington University.

George R. Lyman......Assistant Professor of Botany, Dartmouth College.

B. M. Duggar.........Professor of Plant Physiology, Cornell University.

Ivey F. Lewis..........Professor of Biology, Randolph-Macon College.

Lewis Knudson........Instructor in Plant Physiology, Cornell University.

LIBRARY

H. McE. Knower......University of Cincinnati, Librarian.

CHEMICAL SUPPLIES

Oliver S. Strong......College of Physicians and Surgeons, New York City, Chemist.

G. M. Gray............Curator of Supply Department.
Thomas M. Douthart
and John J. Morton..Collectors in Zoölogy.
J. M. Irwin............Collector in Botany, Dartmouth College.
John Veeder..........Cockswain.

2. INVESTIGATORS AND STUDENTS

1911

INVESTIGATORS—OCCUPYING ROOMS.

ZOÖLOGY

Abbott, James Francis, Professor of Zoölogy, Washington University.

Addison, W. H. F., Demonstrator of Histology and Embryology, University of Pennsylvania.

Bartelmez, George W., Associate in Anatomy, University of Chicago.

Beckwith, Cora J., Instructor in Biology, Vassar College.

Budington, Robert A., Associate Professor of Zoölogy, Oberlin College.

Calkins, Gary N., Professor of Protozoölogy, Columbia University.

Chambers, Robert, Columbia University.

Clapp, Cornelia M., Professor of Zoölogy, Mount Holyoke College.

Conklin, E. G., Professor of Zoölogy, Princeton University.

Craig, Wallace, Professor of Philosophy, University of Maine.

Curtis, W. C., Professor of Zoölogy, University of Missouri.

Dodds, Gideon S., Instructor of Zoölogy, University of Missouri.

Drew, Gilman A., Assistant Director, Marine Biological Laboratory, Woods Hole, Mass.

Dunn, Elizabeth Hopkins, Instructor in Anatomy, University of Chicago.

Fox, Henry, Professor of Biology, Ursinus College, Collegeville, Pa.

Goldfarb, A. J., Instructor, College of the City of New York.

Harvey, Basil C. H., Assistant Professor of Anatomy, University of Chicago.

Harvey, E. Newton, Instructor in Physiology, Princeton University.

Hogue, Mary J., Instructor in Zoölogy, Mount Holyoke College.

Kelley, Frank J., Assistant in Experimental Breeding, University of Wisconsin.

Kellicott, William E., Professor of Biology, Goucher College.

Knower, H. McE., Professor of Anatomy, University of Cincinnati.

Lefevre, George, Professor of Zoölogy, University of Missouri.

Lillie, Frank R., Professor of Embryology, University of Chicago.

Lyon, Mary B., Instructor in Zoölogy, Mount Holyoke College.

McClung, C. E., Professor of Zoölogy, University of Kansas.

Mayer, A. G., Director, Department of Marine Biology, Carnegie Institution.

Montgomery, T. H., Jr., Professor of Zoölogy, University of Pennsylvania.

Morgan, T. H., Professor of Experimental Zoölogy, Columbia University.

Packard, Charles, Assistant in Zoölogy, Columbia University.

Paton, Stewart, Lecturer in Biology, Princeton University.

Patten, William, Professor of Biology, Dartmouth College.

Patterson, J. T., Adjunct Professor of Zoölogy, University of Texas.

Quackenbush, L. S., 27 West 73d Street, New York City.

Rea, Paul M., Professor of Biology, College of Charleston.

Scott, John W., Westport High School, Kansas City, Mo.

Strong, Oliver S., Instructor in Anatomy, College of Physicians and Surgeons, New York City.

Whitney, D. D., Associate Professor of Zoölogy, Wesleyan University, Middletown, Conn.

Wieman, H. L., Assistant Professor of Zoölogy, University of Cincinnati.

Wildman, E. E., Professor of Zoölogy, Central High School, Philadelphia, Pa.

Wilson, E. B., Professor of Zoölogy, Columbia University.

Woodruff, L. L., Assistant Professor of Biology, Yale University.

PHYSIOLOGY

Amberg, Samuel, Associate Professor of Pediatrics, Johns Hopkins University.

Bancroft, Frank W., Associate, Rockefeller Institute for Medical Research, New York City.

Beutner, Reinhard, Assistant, Rockefeller Institute for Medical Research, New York City.

Bradley, H. C., Assistant Professor of Physiological Chemistry, University of Wisconsin.

Donaldson, H. H., Professor of Neurology, Wistar Institute of Anatomy and Biology.

Ferguson, J. S., Assistant Professor of Histology, Cornell University Medical School.

Glaser, O. C., Assistant Professor of Zoology, University of Michigan.

Lillie, R. S., Instructor in Physiological Zoology, University of Pennsylvania.

Loeb, Jacques, Rockefeller Institute for Medical Research, New York City.

Mathews, A. P., Professor of Physiological Chemistry, University of Chicago.

Mathews, Samuel A., Assistant Professor of Experimental Therapeutics, University of Chicago.

Meigs, E. B., Fellow in Zoölogy, Wistar Institute of Anatomy and Biology.

Newman, H. H., Associate Professor of Zoology, University of Chicago.

Pike, Frank W., Instructor in Physiology, University of Chicago.

Rogers, Charles G., Professor of Physiology, Syracuse University.

Spaulding, E. G., Assistant Professor of Philosophy, Princeton University.

Wasteneys, Hardolph, Assistant, Rockefeller Institute for Medical Research, New York City.

BOTANY

Derick, Carrie M., Assistant Professor of Botany, McGill University.

Duggar, B. M., Professor of Plant Physiology, Cornell University.

Knudson, Lewis, Instructor in Plant Physiology, Cornell University.

Lewis, Ivey F., Professor of Biology, Randolph-Macon College.

Lyman, George R., Assistant Professor of Botany, Dartmouth College.

Moore, George T., Professor of Botany, Washington University, St. Louis, Mo.

Osterhout, W. J. V., Assistant Professor of Botany, Harvard University.

Thomas, Mason B., Professor of Botany, Wabash College.

OCCUPYING TABLES

ZOÖLOGY

Abbott, Margaret B., Bennett School, Millbrook, New York.

Allyn, Harriet M., Fellow in Zoölogy, University of Chicago.

Browne, Ethel N., Graduate Student, Columbia University.

Davis, Sarah Ellen, 512 West 132d Street, New York City.
Dungay, Neil S., Professor of Biology, Carleton College, Northfield, Minn.
Eddy, Milton W., Northwestern University, Evanston, Ill.,
Ennis, Agnes, 453 Convent Avenue, New York City.
Just, E. E., Instructor in Biology, Howard University.
Mackenzie, Mary D., Associate Professor of Biology, Western College, Oxford, Ohio.
Spencer, Henry J., Graduate Student, Columbia University.
Sink, Emory W., Assistant in Zoölogy, University of Michigan.
Wallace, Edith M., Columbia University.

PHYSIOLOGY

Morse, Max W., Professor of Biology, Trinity College, Hartford, Conn.
Tashiro, Shiro, Student, University of Chicago.

STUDENTS

1911

INVERTEBRATE ZOÖLOGY

Baird, Grace J., 608 Mathews Avenue, Urbana, Ill.
Boland, Mildred, Western College, Oxford, Ohio.
Brown, Virginia R., 341 Prescott Street, Toledo, Ohio.
Butterweck, Joseph R., 1729 Turner Street, Allentown, Pa.
Copenhaver, Nat H., Bristol, Tenn.
Dexter, John S., Professor of Biological Sciences, Northland College, Ashland, Wisconsin.
Dodd, Helen N., 25 Appleton Place, Glen Ridge, N. J.
Dodd, Jennie S., 333 West 77th Street, New York City.
Ferguson, Albert B., University of Maine, Orono, Maine.
Flanigen, Ruth, William Penn High School, Philadelphia.
Gibbs, Ethel C., Carthage, New York.
Jenner, Edwin A., Professor of Biology, Simpson College, Indianola, Iowa.
Johnson, Bertha T., Oberlin College, Oberlin, Ohio.
Lynch, Frances M., Goucher College, Baltimore, Md.
Nute, Helen E., 914 Highland Avenue, Fall River, Mass.
Murray, Marjorie F., Bryn Mawr College.
Rowe, Elizabeth A., 1835 E. Baltimore Street, Baltimore, Md.
Schiel, Dora E., Mount Holyoke College.
Smiley, Carolyn D., Mount Holyoke College.
Snyder, Helen C., 42 Madison Avenue, Jersey City, N. J.
Spray, Ruth G., University of Kansas.
Steffen, Anna E., Oberlin College.
Trenor, Albert D., 142 E. 62d Street, New York City.
Warren, Helen F., 16 Brent Street, Dorchester, Mass.
White, Esther L., Meridian College, Meridian, Texas.

EMBRYOLOGY

Altenburg, Edgar, 2803 Third Avenue, New York City.
Anderegg, Louis T., Oberlin College.
Box, Cora M., Instructor, University of Cincinnati.
Burke, Edmund J., Instructor in Biology, Holy Cross College, Worcester, Mass.
Cattell, McKeen, Garrison, New York.
Collett, Mary E., 915 North 5th Street, Atchinson, Kansas.
Cooper, Georgia M., 13 Highland Avenue, Auburn, Maine.
Glascock, Hardin R., Ohio Wesleyan University, Delaware, Ohio.
Glaser, Rudolph W., University of Michigan.
Hoge, Mildred A., Western High School, Baltimore, Md.
Kelly, James P., Black Rock Avenue, Unionport, New York City.
Morriss, Margaret, 53 Edgehill Road, New Haven, Conn.

Muller, Hermann J., 562 West 150th Street, New York City.
Painter, Theophilus S., Yale University.
Patten, Hazel, 1719 McCulloh Street, Baltimore, Md.
Richards, Ethel L., Washington University, St. Louis, Mo.
Saenz, Moises, Washington and Jefferson College, Washington, Pa.
Schwitalla, Alphonse M., Instructor in Biology, St. Louis University.
Wheeler, Theodora, Fairfield, Conn.
Walker, George, Associate in Surgery, Johns Hopkins University.
Northrup, John H., 500 Washington Avenue, Yonkers, New York.

PHYSIOLOGY

Bigney, Andrew J., Professor of Biology and Geology, Moores Hill College, Indiana.
Carroll, Albert H., "Evergreen," Hampden, Baltimore, Md.
Ickes, Margaret, Smith College, Northampton, Mass.
Kellersberger, Eugene R., University of Texas.
Norcross, Katharine, University of Chicago.
Oliver, Wade W., University of Michigan.

BOTANY

Bosson, Richard M., Wabash College.
Carey, Cornelia L., Box 956, Orange, N. J.
Curtis, Otis F., 163 North Main Street, Oberlin, Ohio.
Dawson, Ava B., 97 Mountfort Street, Boston, Mass.
Faust, Ernest C., Oberlin College.
Gross, Beatrix H., Normal College, New York City.
Hill, Albert F., 192 South Main Street, Attleboro, Mass.
Jennison, Harry M., Auburn, Mass.
Kenoyer, Leslie A., Leander Clark College, Toledo, Iowa.
Noble, Arlyle, Smith College.
Richards, Annabella E., Ventnoe City, N. J.
Welsh, Carlton K., Dartmouth College.
Pennell, Francis W., Harrison Fellow in Botany, University of Pennsylvania.

3. TABULAR VIEW OF ATTENDANCE

	1908	1909	1910	1911
INVESTIGATORS—Total	52	66	62	82
Occupying Rooms				
Zoölogy	32	39	33	42
Physiology	6	7	9	18
Botany	8	4	9	8
Occupying Tables				
Zoölogy	6	14	5	12
Physiology		2	2	2
Botany			1	
STUDENTS—Total	48	63	64	65
Zoölogy	19	36	31	26
Embryology	15	12	10	20
Physiology	3	9	5	6
Botany	11	6	17	13
INSTITUTIONS REPRESENTED				
By Investigators	25	27	26	37
By students	26	20	24	31
SCHOOLS AND ACADEMIES REPRESENTED				
By investigators	2	3	5	3
By students	3	11	6	9

4. SUBSCRIBING INSTITUTIONS, 1911

Bryn Mawr College.
Columbia University.
Dartmouth College.
Goucher College.
Lucretia Crocker Scholarship.
Mount Holyoke College.
Northwestern University.
Oberlin College.
Princeton University.
Rockefeller Institute for Medical Research.
Sheffield Scientific School of Yale University.
Smith College.
Syracuse University.
Trinity College, Hartford, Conn.
University of Chicago.
University of Cincinnati.
University of Illinois.
University of Kansas.
University of Michigan.
University of Pennsylvania.
Vassar College.
Washington University Alumni Association.
Western College for Women.
Wellesley College.
Wistar Institute of Anatomy and Biology.
Scholarship of $100. Supported by a friend of the Laboratory since 1898.

5. EVENING LECTURES, 1911

Gary N. Calkins	"The Scope of Protozoölogy"	June 30.
G. H. Parker	"Some Recent Work on Animal Reactions to Colored Light"	July 3.
T. H. Morgan	"What is the Mechanism of Mendelian Segregation in the Germ Cells?"	July 7.
I. F. Lewis	"Alternation of Generations and Periodicity in the Marine Algæ"	July 11.
Simon Flexner	"The Biological Basis of the Treatment of Disease"	July 14
Wallace Craig	"Why Do Birds Sing?"	July 18.
J. McKeen Cattell	"Science and Democracy"	July 21.
Bradley M. Davis	"The Synthesis and Behavior of Some Hybrids that Resemble *Œnothera Lamarckiana*"	July 25.
Jacques Loeb	"The Life-Preserving Action of Salts"	July 28.
E. G. Spaulding	"Bergson's Creative Evolution"	Aug. 4.
W. M. Wheeler	"Insect Parasitism and Its Peculiarities"	Aug. 8.

6. MEMBERS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

AUGUST 8, 1911

Life Members

Allis, Mr. Edward Phelps, Jr., Palais Carnoles, Menton, France.

Andrews, Mrs. Gwendolen Foulke, 821 St. Paul St., Baltimore, Md.

Billings, Mr. R. C., 66 Franklin Street, Boston, Mass.

Carey, Mr. Arthur Astor, Fayerweather Street, Boston, Mass.

Clarke, Prof. S. F., Williams College, Williamstown, Mass.

Conklin, Dr. E. G., Princeton University, Princeton, New Jersey.

Crane, Mr. C. R., 2559 Michigan Boulevard, Chicago, Ill.

Davis, Major Henry M., Syracuse, New York.

Endicott, William, Jr., 31 Beacon St., Boston, Mass.

Evans, Mrs. Glendower, 12 Otis Place, Boston, Mass.

Farlow, Prof. W. G., Harvard University, Cambridge, Mass.

Fay, Miss S. B., 88 Mt. Vernon Street, Boston, Mass.

Folsom, Miss Amy, 88 Marlborough St., Boston, Mass.

Foot, Miss Katharine, 80 Madison Avenue, New York City.

Gardiner, Miss Eugenia, 15 West Cedar Street, Boston, Mass.

Hammond, Mr. G. W., Hotel Hamilton, Boston, Mass.

Hannaman, Mr. Charles E., 103 First Street, Troy, New York.

Harrison, Provost C. C., University of Pennsylvania, Philadelphia, Pa.

Herter, Dr. C. A., 819 Madison Avenue, New York City.

Higginson, Mr. Henry L., 191 Commonwealth Avenue, Boston, Mass.

Jackson, Miss M. C., 88 Marlborough Street, Boston, Mass.

Jackson, Mr. Charles C., 24 Congress Street, Boston, Mass.

Kennedy, Mr. George G., 284 Warren Street, Roxbury, Mass.

Kidder, Mr. C. G., 27 William Street, New York City.

Kidder, Mr. Nathaniel T., Milton, Mass.

KING, MR. CHARLES A.

LEE, MRS. FREDERIC S., 279 Madison Avenue, New York City.

LOWELL, MR. A. LAWRENCE, 171 Marlborough Street, Boston, Mass.

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MASON, MISS IDA M., 1 Walnut Street, Boston, Mass.

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